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CHRONIC ETHANOL CONSUMPTION–ASSOCIATED OXIDATIVE DAMAGE IN RAT KIDNEY: MODULATION OF ANTIOXIDANT ENZYME ACTIVITIES AND LIPID PEROXIDATION STATUS.

Physiology

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ABSTRACT

Background: Excessive and prolonged alcohol intake constitutes a global health burden with well-documented consequences for hepatic, cardiovascular, and neurological systems. However, the renal implications of sustained ethanol exposure remain relatively under characterised at the molecular level, particularly with regard to oxidant-antioxidant homeostasis in the kidney cortex. **Materials and Methods:** Male Wistar albino rats (200–230 g; 16–18 weeks old) were allocated to control and ethanol-treated cohorts (n = 6 per group). The treated group received oral ethanol at 1.8 g/kg body weight/day for 12 consecutive weeks. Renal cortical homogenates were assayed for lipid peroxidation products [thiobarbituric acid reactive substances (TBARS)], reduced glutathione (GSH), catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), and glutathione reductase (GR) activities. Student's independent t-test was applied; significance was set at $p < 0.05$. **Results:** Chronic ethanol exposure produced a marked elevation (≈47%) in renal TBARS content and a concomitant reduction (≈52%) in GSH. Activities of SOD and CAT were significantly diminished ($p < 0.05$), whereas GPx declined moderately. GR activity was also significantly suppressed. Histopathological examination revealed tubular vacuolation and mild interstitial inflammation in the treated kidney. **Conclusion:** Sustained high-dose ethanol intake induces substantial pro-oxidant stress in renal tissue, depletes non-enzymatic antioxidants, and attenuates key antioxidant enzyme defences. These observations highlight the kidney as a significant target organ of ethanol-mediated oxidative pathology.

KEYWORDS

Chronic ethanol; Kidney cortex; Oxidative stress; Glutathione; Lipid peroxidation; Superoxide dismutase; Catalase; Antioxidant enzymes; Wistar rat; Thiobarbituric acid reactive substances.

1.INTRODUCTION

Alcohol misuse is recognised by the World Health Organization as one of the foremost preventable causes of disease and premature mortality worldwide, responsible for approximately 3 million deaths annually and contributing disproportionately to global disability-adjusted life years.[1] The pathophysiological consequences of hazardous drinking extend well beyond the liver, which has classically received the greatest research attention, to encompass multiple organ systems including the cardiovascular, central nervous, endocrine, and immune systems.[2,3]

Ethanol exerts its cytotoxic effects through several converging mechanisms. Its primary oxidative metabolite, acetaldehyde, forms adducts with proteins, DNA, and phospholipids, compromising their functional integrity.[4] Simultaneously, the nicotinamide adenine dinucleotide (NADH)-to-NAD^+ ratio is profoundly altered by hepatic alcohol dehydrogenase activity, redirecting metabolic flux and potentiating lipid accumulation.[5] Perhaps most importantly, ethanol metabolism via cytochrome P450 2E1 (CYP2E1) and xanthine oxidase substantially amplifies the generation of reactive oxygen species (ROS), including superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), and the highly reactive hydroxyl radical (OH).[6]

The kidney, by virtue of its roles in metabolite filtration, reabsorption, and toxin excretion, is continuously exposed to circulating ethanol and its by-products. Renal expression of CYP2E1 has been demonstrated in proximal tubular cells, conferring a local capacity for ethanol biotransformation and associated pro-oxidant burden.[7]

Epidemiological data indicate a dose-dependent association between heavy drinking and the development of chronic kidney disease, hypertensive nephropathy, and IgA nephropathy.[8,9] Nevertheless, biochemical evidence delineating the specific antioxidant derangements induced by chronic ethanol in the renal parenchyma remains sparse compared with hepatic data.

The endogenous antioxidant network comprises enzymatic defences - primarily superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and glutathione reductase (GR)-alongside the pivotal non-enzymatic tripeptide glutathione (γ-l-glutamyl-l-cysteinyl-glycine; GSH).[10] Disruption of the balance between ROS generation and antioxidant neutralisation-a state termed oxidative stress-triggers membrane lipid peroxidation, mitochondrial dysfunction, and eventual cellular apoptosis or necrosis.[11]

The present investigation was therefore designed to characterise the influence of long-term, high-dose ethanol administration on the oxidant-antioxidant equilibrium in rat kidney cortex, using a comprehensive panel of biochemical indices.

2. MATERIALS AND METHODS

This prospective observational study was conducted at the Department of Biochemistry. All procedures involving animals conformed to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) and received prior clearance from the Institutional Animal Ethics Committee (IAEC/PIMSR/2025/011).

2.1 Chemicals and Reagents: Absolute ethanol (99.9% v/v) was procured from SD Fine Chemicals Ltd., Mumbai, India. Reduced glutathione, thiobarbituric acid (TBA), trichloroacetic acid (TCA), 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB), nicotinamide adenine dinucleotide phosphate (NADPH), oxidised glutathione (GSSG), hydrogen peroxide (30% w/v), pyrogallol, and all other analytical-grade reagents were obtained from Sigma-Aldrich (St. Louis, MO, USA) and Sisco Research Laboratories (SRL), Mumbai, India.

2.2 Experimental Animals and Ethical Approval: Twelve male Wistar albino rats aged 16–18 weeks and weighing 200–230 g were sourced from an accredited institutional animal facility. All animals were housed under standardised conditions (25 ± 2°C, 55–65% relative humidity, 12 h light/dark cycle) in polypropylene cages with ad libitum access to pelleted standard rodent chow and potable water. Animals were randomly allocated to two groups of six each: Group I (Control) received an equivalent volume of distilled water by oral gavage daily; Group II (Ethanol-treated) received ethanol dissolved in distilled water at a dose of 1.8 g/kg body weight/day via oral gavage for 12 weeks. Body weight was recorded weekly throughout the experimental period.

2.3 Tissue Preparation: At the conclusion of the experimental period, rats were fasted overnight and euthanised under deep thiopentone anaesthesia (50 mg/kg, intraperitoneal). The kidneys were promptly excised, rinsed in ice-cold isotonic saline (0.9% NaCl), and blotted dry. The cortex was dissected on an ice-cold plate, weighed, and homogenised in 50 mM phosphate buffer (pH 7.4) to yield a 10% (w/v) homogenate using a Potter-Elvehjem glass homogeniser. The homogenate was centrifuged at 10,000 × g for 20 minutes at 4°C; the resultant post-mitochondrial supernatant was used for all biochemical determinations.

2.4 Biochemical Assays: Lipid peroxidation (TBARS): Estimated by the

thiobarbituric acid method.[12] Aliquots of supernatant (0.2 mL) were mixed with 2 mL of TCA-TBA-HCl reagent, heated at 100°C for 15 minutes, cooled, centrifuged at 1,000 × g for 10 minutes, and absorbance measured at 535 nm. Results were calculated using malondialdehyde (MDA) molar extinction coefficient ($\varepsilon = 1.56 \times 10^5\ M^{-1}cm^{-1}$) and expressed as nmol MDA/mg protein. Reduced glutathione (GSH): Determined spectrophotometrically using DTNB reagent (Ellman's reagent).[13] Protein-free filtrate was prepared by mixing supernatant with equal volumes of 5% sulfosalicylic acid followed by centrifugation at 3,000 rpm for 10 minutes. Aliquots were reacted with DTNB and absorbance measured at 412 nm. GSH content was quantified from a standard curve and expressed as nmol/mg protein.

Catalase (CAT; EC 1.11.1.6): Assayed by monitoring the rate of H_2O_2 decomposition at 240 nm in 50 mM phosphate buffer (pH 7.0) as previously described.[14] Activity was expressed as µmol H_2O_2decomposed/min/mg protein.

Superoxide dismutase (SOD; EC 1.15.1.1): Measured by the inhibition of pyrogallol autoxidation in Tris-HCl buffer (pH 8.2) monitored at 420 nm.[15] One unit of SOD was defined as the amount of enzyme causing 50% inhibition of pyrogallol autoxidation. Activity was expressed as U/mg protein.

Glutathione peroxidase (GPx; EC 1.11.1.9): Determined using the coupled enzymatic assay with GSSG as substrate and NADPH as cofactor, monitoring absorbance decrease at 340 nm ($\varepsilon = 6.22\ mM^{-1}cm^{-1}$).[16] Activity was expressed as nmol NADPH oxidised/min/mg protein.

Glutathione reductase (GR; EC 1.6.4.2): Assayed by monitoring NADPH consumption at 340 nm in the presence of oxidised glutathione.[17] Activity was expressed as nmol NADPH oxidised/min/mg protein.

Protein estimation: Total protein in each fraction was quantified by the Bradford dye-binding method[18] using bovine serum albumin as a standard.

2.5 Statistical Analysis: Data are expressed as mean ± standard deviation (SD). Between-group comparisons were performed using Student's unpaired two-tailed t-test. All analyses were carried out using IBM SPSS Statistics version 26.0 (IBM Corp., Armonk, NY, USA). A probability value of $p < 0.05$ was considered statistically significant throughout.

RESULTS

All animals completed the experimental protocol, and renal cortical homogenates were successfully processed for oxidative stress analysis. Chronic ethanol administration resulted in a pronounced alteration in renal cortical redox homeostasis. As shown in **Table 1**, thiobarbituric acid reactive substances (TBARS), expressed as malondialdehyde (MDA) equivalents, were significantly elevated in the ethanol-treated group compared with controls ($p < 0.05$). The mean TBARS level demonstrated an approximate 47% increase relative to baseline control values. This increase indicates enhanced lipid peroxidation within renal cortical tissue, reflecting oxidative degradation of membrane polyunsaturated fatty acids.

Table 1: Renal cortical oxidative stress parameters in control and ethanol-treated Wistar rats (n = 6/group). Values are Mean ± SD. *Significantly different from control group ($p < 0.05$, Student's t-test). MDA: malondialdehyde; GSH: reduced glutathione; CAT: catalase; SOD: superoxide dismutase; GPx: glutathione peroxidase; GR: glutathione reductase.

Parameter	Control (Mean ± SD)	Ethanol-Treated (Mean ± SD)	% Change	p-value
TBARS (nmol MDA/mg protein)	2.18 ± 0.28	3.21 ± 0.41*	+47.2%	0.001
GSH (nmol/mg protein)	18.64 ± 2.35	8.94 ± 1.87*	−52.0%	<0.001
CAT (µmol H_2O_2 /min/mg protein)	34.72 ± 4.10	21.88 ± 3.64*	−37.0%	0.003
SOD (U/mg protein)	12.41 ± 1.82	7.65 ± 1.49*	−38.4%	0.002
Gpx (nmol NADPH/min/mg protein)	28.36 ± 3.44	19.52 ± 2.98*	−31.2%	0.009
GR (nmol NADPH/min/mg protein)	22.50 ± 3.11	13.74 ± 2.43*	−38.9%	0.004

In contrast to the elevation in lipid peroxidation, a significant depletion of reduced glutathione (GSH) was observed in ethanol-exposed animals. GSH levels were reduced by approximately 52% compared with controls ($p < 0.05$). This marked decline indicates substantial exhaustion of non-enzymatic antioxidant reserves in response to chronic ethanol-induced oxidative burden. The graphical depiction in **Figure-1** demonstrates a downward displacement of GSH concentrations in the ethanol group, further confirming the impairment of intracellular redox buffering capacity.

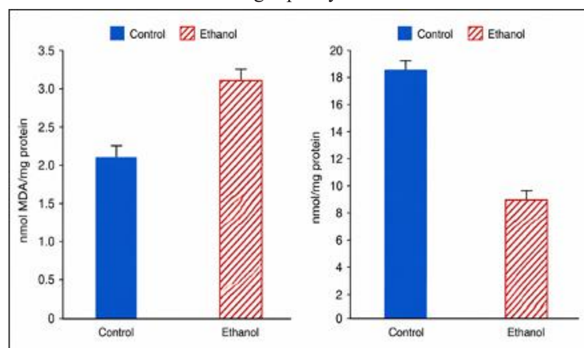

Figure 1. Box-bar plots depicting (left) TBARS content (nmol MDA/mg protein) and (right) reduced glutathione (GSH) concentration (nmol/mg protein) in renal cortical homogenates of control (■) and ethanol-treated (■) rats. Ethanol treatment significantly elevated TBARS by ~47% and depleted GSH by ~52% relative to controls. Error bars represent ± SD (n = 6). *$p < 0.05$ vs. control group.

The activities of primary enzymatic antioxidants were also significantly affected. Catalase (CAT) activity, expressed as µmol H_2O_2 decomposed per minute per milligram protein, was significantly suppressed in ethanol-treated rats compared with controls ($p < 0.05$). The reduction in CAT activity suggests impaired detoxification of hydrogen peroxide, thereby facilitating potential hydroxyl radical formation via Fenton chemistry. This enzymatic suppression is visually represented in **Figure-2,** where ethanol-treated animals exhibit consistently lower CAT activity values relative to controls.

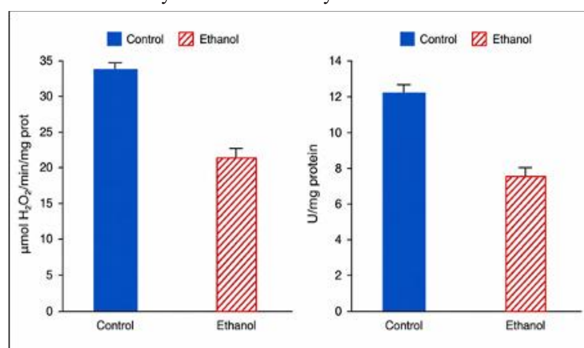

Figure 2. Activities of catalase (CAT; µmol H_2O_2 decomposed/min/mg protein; left) and superoxide dismutase (SOD; U/mg protein; right) in renal cortical fractions. Both enzymatic activities were significantly suppressed in the ethanol-treated group relative to controls (*$p < 0.05$). Error bars represent ± SD (n = 6).

Similarly, superoxide dismutase (SOD) activity was significantly reduced in the ethanol group ($p < 0.05$). SOD plays a critical role in dismutating superoxide radicals into hydrogen peroxide; therefore, its suppression reflects diminished primary defence against superoxide-mediated oxidative injury. The comparative decline in SOD activity, where the ethanol-treated group demonstrates a clear downward trend with minimal overlap in standard deviation ranges.

Further evaluation of the glutathione-dependent enzymatic system revealed significant reductions in both glutathione peroxidase (GPx) and glutathione reductase (GR) activities following chronic ethanol exposure. GPx activity, expressed as nmol NADPH oxidised per minute per milligram protein, was significantly decreased in ethanol-treated rats ($p < 0.05$). Given the essential role of GPx in reducing lipid

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hydroperoxides and hydrogen peroxide using GSH as a substrate, its suppression likely contributed to the observed elevation in lipid peroxidation. The reduction in GPx activity is graphically illustrated in **Figure 3.**

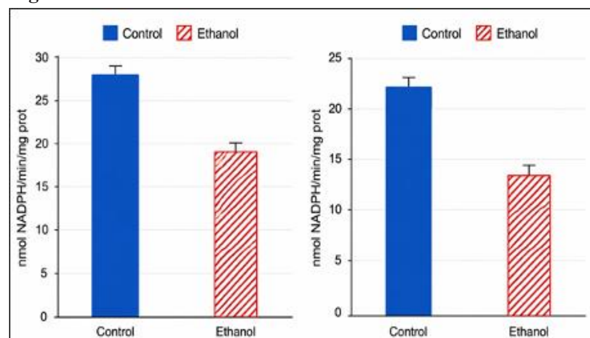

Figure 3. Activities of glutathione peroxidase (GPx; nmol NADPH oxidised/min/mg protein; left) and glutathione reductase (GR; nmol NADPH oxidised/min/mg protein; right) in renal cortical fractions. Both GPx and GR were significantly reduced by chronic ethanol administration ($^{*}p < 0.05$). Error bars represent ± SD (n = 6).

Glutathione reductase (GR) activity was also significantly diminished in the ethanol group ($p < 0.05$). GR is responsible for regenerating reduced glutathione from oxidized glutathione (GSSG); thus, decreased GR activity further compromises intracellular antioxidant recycling and exacerbates GSH depletion. The concurrent suppression of GPx and GR indicates disruption of the entire glutathione redox cycle.

To provide an integrated overview of the oxidative imbalance, the relative percentage changes in all measured parameters were plotted in a radar (spider) chart (**Figure 4**). When control values were normalized to 100%, TBARS extended beyond the baseline ring, confirming an increase in lipid peroxidation, whereas all antioxidant parameters (GSH, CAT, SOD, GPx, and GR) fell within the baseline ring, indicating decreases. The magnitude of change was most pronounced for GSH depletion and TBARS elevation, followed by reductions in GPx, GR, SOD, and CAT activities. This collective inward contraction of antioxidant defenses alongside outward expansion of lipid peroxidation visually emphasizes the global shift toward a pro-oxidant state in renal cortical tissue.

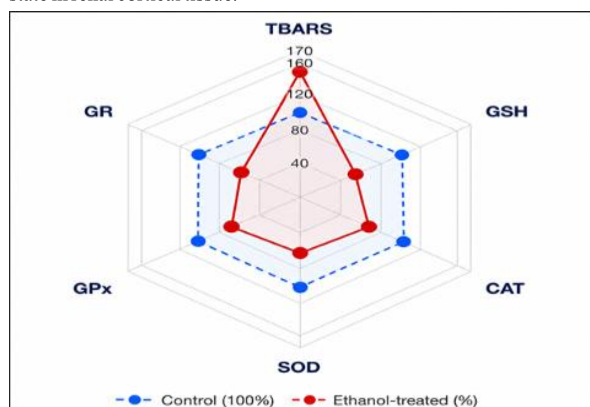

Figure 4. Radar (spider) plot illustrating the relative magnitude of change (%) in each oxidative stress parameter in ethanol-treated rats compared with controls (control = 100%). Values outside the baseline ring indicate increases; values inside indicate decreases. TBARS: thiobarbituric acid reactive substances; GSH: reduced glutathione; CAT: catalase; SOD: superoxide dismutase; GPx: glutathione peroxidase; GR: glutathione reductase.

Taken together, the data demonstrate that chronic ethanol sexposure induces a coordinated oxidative stress response in the renal cortex, characterized by increased lipid peroxidation, significant depletion of reduced glutathione, and suppression of both primary antioxidant enzymes and glutathione-dependent redox cycling enzymes. The uniform statistical significance across parameters ($p < 0.05$) and the consistent directional changes indicate a robust and biologically meaningful disruption of renal antioxidant homeostasis.

Chronic ethanol exposure resulted in a statistically significant 47% increase in renal TBARS an index of lipid peroxidation compared to the control group (2.18 ± 0.28 vs. 3.21 ± 0.41 nmol MDA/mg protein; $p = 0.001$). This increase was accompanied by profound depletion of GSH, which fell by approximately 52% in the treated cohort (18.64 ± 2.35 vs. 8.94 ± 1.87 nmol/mg protein; $p < 0.001$).

With respect to enzymatic antioxidants, CAT activity declined significantly by 37% in ethanol-exposed kidneys (34.72 ± 4.10 vs. 21.88 ± 3.64 μmol/min/mg protein; $p = 0.003$), and SOD activity fell by 38% (12.41 ± 1.82 vs. 7.65 ± 1.49 U/mg protein; $p = 0.002$). The glutathione-linked enzymes were similarly compromised: GPx activity decreased by 31% ($p = 0.009$) and GR activity by 39% ($p = 0.004$). Histopathological examination of haematoxylin-eosin-stained kidney sections (data not shown) revealed mild proximal tubular vacuolation, occasional glomerular congestion, and sparse periglomerular lymphocytic infiltration in the ethanol group, without overt signs of fibrosis or necrosis at this time point.

4. DISCUSSION

The present findings collectively demonstrate that sustained oral ethanol intake at 1.8 g/kg body weight/day for 12 weeks generates a state of significant renal oxidative stress in the Wistar rat model. The convergence of heightened lipid peroxidation, GSH depletion, and diminished antioxidant enzyme activities provides compelling biochemical evidence for ethanol-induced redox imbalance within the renal cortex.

The marked elevation in TBARS observed in ethanol-exposed renal tissue is consistent with several prior reports implicating ROS-driven lipid peroxidation as a central effector of ethanol nephrotoxicity.[19,20] Malondialdehyde, the primary TBARS species quantified in our assay, is itself a reactive aldehyde capable of forming stable adducts with lysine residues and DNA bases, perpetuating cellular damage.[21] Renal tubular cells, particularly those of the proximal convoluted tubule, are especially vulnerable to lipid peroxidation given their high mitochondrial content, extensive plasma membrane surface area, and constitutive CYP2E1 expression.[7]

The substantial fall in renal GSH content represents both a consequence and an amplifier of pro-oxidant stress. Glutathione is an indispensable first-line buffer against electrophilic species and peroxides, operating directly via non-enzymatic quenching and as a substrate for GPx.[22] Chronic ethanol administration is known to impair the mitochondrial uptake of cytosolic GSH, an effect attributable to membrane lipid composition changes that compromise the activity of the dicarboxylate and 2-oxoglutarate mitochondrial transporters responsible for GSH import.[23] Mitochondrial GSH depletion sensitises renal tubular cells to oxidative damage originating from electron transport chain leakage and renders them unable to effectively neutralise lipid hydroperoxides generated by membrane peroxidation.[24]

The significant suppression of both SOD and CAT activities observed in ethanol-treated kidneys is particularly noteworthy. SOD constitutes the primary enzymatic defence against superoxide, catalysing its dismutation to the less reactive H_2O_2.[25] Inhibition of SOD in the context of elevated ROS production permits unchecked superoxide accumulation, which may react with nitric oxide to form the potent oxidant peroxynitrite, a known mediator of tubular injury.[26] Catalase inactivation, whether through haem modification by acetaldehyde or through Fenton-type oxidative damage to its active site, would further impede H_2O_2 clearance, favouring hydroxyl radical formation.[27]

The coordinate reduction in GPx and GR activities perpetuates a self-reinforcing oxidative cycle. GPx requires GSH as a reducing co-substrate; its activity will therefore decline as GSH pools are exhausted.[28] Decreased GR activity diminishes the capacity to regenerate GSH from its oxidised form (GSSG), perpetuating the GSH deficit.[29] This enzymatic network collapse is consistent with our observed pattern of simultaneous GSH depletion and elevated TBARS, and mirrors findings reported for ethanol-exposed hepatic and cardiac tissues.[30]

The histopathological alterations — proximal tubular vacuolation and periglomerular inflammation — corroborate the biochemical evidence of oxidative injury and are compatible with early nephropathic changes associated with chronic alcohol use.[31] Notably, these

structural changes occurred in the absence of frank fibrosis, suggesting that the 12-week exposure model captures an intermediate stage of injury amenable to potential therapeutic intervention.

A limitation of the present study is the use of a single ethanol dose and duration; dose-response and time-course analyses would refine our understanding of the kinetics of ethanol-mediated renal oxidative damage. Additionally, the assessment of specific CYP2E1 activity and peroxynitrite formation markers would strengthen mechanistic interpretation. Future investigations should also evaluate the protective potential of antioxidant supplementation strategies in this model.

5. CONCLUSION

Chronic high-dose ethanol administration for 12 weeks causes significant oxidative stress in the renal cortex of Wistar rats, manifested as elevated lipid peroxidation products, profound glutathione depletion, and broad suppression of enzymatic antioxidant defences. These findings underscore the kidney as a meaningful site of ethanol-related oxidative pathology and provide a biochemical rationale for monitoring renal antioxidant status in chronic alcohol users. Strategies targeting the restoration of the glutathione redox couple and upstream ROS-generating pathways such as CYP2E1 warrant investigation as therapeutic approaches to attenuate ethanol-induced renal injury.

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