ORIGINAL ARTICLES HEPATOLOGY

Time-Dependent Alterations in Liver Oxidative Stress due to Ethanol and Acetaldehyde

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Abstract

Binge drinking is a major public health issue and ethanol-related liver insult may play a major role in the pathology of alcoholic liver disease. However, the degree of oxidative stress, cell death and contribution of acetaldehyde to liver damage over a 24-h period has yet to be determined. Herein, we aimed to investigate the effect of acute alcohol and elevated acetaldehyde levels on hepatic oxidative damage, apoptosis, and anti-oxidant enzyme activity over a 24-h period. Male Wistar rats were divided into four groups and animals were pre-injected (intraperitonially [i.p.]) with either saline (0.15 mol/L) or cyanamide (5-mmol/kg bodyweight), followed by either saline (0.15 mol/L) or ethanol (75-mmol/kg bodyweight). After 2.5, 6 and 24 h, hepatic cytosolic and mitochondrial fractions were analysed for indices of oxidative stress. At 2.5 h, cytosolic glutathione and malondialdehyde levels were significantly reduced and increased, respectively, with alcohol treatment. Caspase-3 activity and cytochrome c levels were increased with alcohol treatment at 24 h. The combination of cyanamide and alcohol treatment at 24 h led to a significant increase in serum alanine aminotransferase levels, and reduced albumin and total protein levels. Furthermore, glutathione peroxidase activity and glutathione reductase activity were significantly decreased and increased, respectively. Finally, superoxide dismutase activity was decreased in cytosol and increased in the mitochondria after cyanamide and ethanol treatment, respectively. This study indicates a complex differential effect of alcohol and acetaldehyde, whereby alcohol toxicity in the mitochondria takes place throughout the 24-h period, but raised acetaldehyde has a further detrimental effect on liver function.

Keywords: acetaldehyde; alcohol; antioxidant; apoptosis; mitochondria; oxidative stress

Introduction

Alcoholic liver disease (ALD) is a major complication of excessive alcohol consumption. Epidemiological studies indicate that the prevalence of binge drinking is mounting among most age groups (1). Alcohol dehydrogenase (ADH) and cytochrome p450 2E1 (CYP2E1) are the main enzymes involved in the oxidative pathways of alcohol metabolism. Reactive oxygen species (ROS) are generated when CYP2E1

oxidises ethanol to acetaldehyde in the endoplasmic reticulum (ER) (2). It is widely documented that chronic ethanol intake causes oxidative liver damage (1) due to a chain reaction of events initiated by ROS formation caused by ethanol metabolism as well as imbalances between elimination and production of ROS and antioxidants (2). These ROS can cause lipid peroxidation, generating products such as malondialdehyde (MDA) and can also trigger an inflammatory response leading to pro-inflammatory cytokine production, mitochondrial dysfunction and apoptosis (2). Alcohol consumption raises acetaldehyde levels (3). Acetaldehyde causes mitochondrial dysfunction, compromising metabolism and perpetuating the accumulation of acetaldehyde (4). Acetaldehyde can also form adducts with cellular proteins, including MDA, leading to an antibody-mediated immune response and induction of hepatocyte apoptosis (2). MDA and acetaldehyde react together forming malondialdehyde–acetaldehyde adducts (MAAs), which have potent pro-inflammatory and pro-fibrogenic properties as well as the generation of antibodies against both MAA and carrier proteins (5). Patients presenting with both mild and severe ALD documented higher levels of acetaldehyde adducts than patients with non-alcoholic fatty liver disease or non-alcoholic hepatitis (6). Production of acetaldehyde via metabolism of ethanol also results in the indirect formation of free radicals and decreases antioxidant defences, resulting in oxidative stress (2).

Glutathione (GSH) and glutathione peroxidase (GPx) play a major role in protection against ROS generation and oxidative stress. Thus, changes to GSH regulation, synthesis and maintenance can lead to cytosolic and mitochondrial oxidative stress (7). Acetaldehyde alone can invoke cell damage, since HepG2 cells exposed to 100-µM acetaldehyde for up to 2 days showed reduced mitochondrial glutathione (mGSH) levels (8). Decrease in mGSH was due to an impairment of the mGSH uptake system. Exposure of isolated mitochondria to acetaldehyde induces oxidative stress impairing mitochondrial function (9). This suggests that mitochondria are unable to replenish their GSH reserves, thus increasing susceptibility to oxidative damage. While there is a clear association between binge drinking and liver disease, there is limited information on the impact of binge drinking on hepatic function in relation to oxidative stress and elevated acetaldehyde levels *in vivo*. A model of binge drinking was utilised to study biomarkers of liver oxidative damage and apoptosis over a 24-h period. In this model, blood alcohol levels attained were 50–60 mM, which were similar to that in binge drinking alcoholics (10). In order to assess the role of acetaldehyde in the induction of tissue damage, cyanamide (an aldehyde dehydrogenase [ALDH] inhibitor) was utilised to block acetaldehyde metabolism prior to high alcohol dosing (11). Time-dependent effects of acute ethanol and acetaldehyde on hepatic oxidative damage and apoptosis were studied.

Methods

Animals

Male Wistar rats (100 g) obtained from Charles River (Bicester, UK) were housed according to good laboratory practice guidelines at the Biological Services Unit of Kings College, London. After 1 week of acclimatisation, weight-matched animals were divided into the following four groups: (i) Group 1—Control saline (0.15-mol/L NaCl), i.p.-treated (n = 6); (ii) Group 2—ethanol (75-mmol/kg bodyweight; equivalent to 3.5 g/kg), i.p.-treated (n = 8); (iii) Group 3—pre-treatment with cyanamide for 30 min (0.5-mmol/kg bodyweight), followed by i.p. saline 0.15 mol/L (n = 6); and (iv) Group 4—pre-treatment for 30 min with ALDH inhibitor cyanamide (0.5-mmol/kg bodyweight), followed by i.p. ethanol, 75-mmol/kg bodyweight (n = 8). Groups 1 and 2 were also pre-treated with 0.15-mol/L saline for 30 min. Upon treatment, food sources were removed, although free access to drinking water continued. After 2.5, 6 and 24 h, animals were sacrificed, their blood samples collected, and the livers excised. Blood was used for the measurement of liver hepatic enzymes. Hepatic cytosol and mitochondria were prepared from pooled livers of two treated animals of each group by differential centrifugation as described previously (12).

Liver function tests

Following sacrifice, blood was immediately collected in heparin funnels and allowed to clot at room temperature for 10 min. Blood was centrifuged (2000 g, 10 min, 4°C) and the serum was collected and stored at -80°C until analysis. The analytes were measured as described previously (13). Total protein, albumin and globulin levels and activities of the enzymes aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were measured.

Glutathione levels

Glutathione levels were determined using an assay adapted from Tietze (14) based on the conversion of 5'5'-dithio-bis-2-nitrobenzoic acid (DNTB) to the yellow-coloured 5-thio-2-nitrobenzoic acid (TNB) by enzyme glutathione reductase (GR). Cytosolic or mitochondrial protein, 25 or 50 µg, was added to the reaction buffer (100-mM sodium phosphate, 1-mM ethylenediaminetetraacetic acid [EDTA], 0.5-mM 5,5′-dithiobis(2-nitrobenzoic acid) [DTNB/Ellman's reagent], 0.175-mM nicotinamide adenine

dinucleotide phosphate [NADPH], and 1.7-U/mL GR) and the absorbance was measured at 412 nm after 25 min.

Malondialdehyde levels

Levels of MDA were measured using a colorimetric thiobarburitic acid reactive substances assay adapted from Bar-Or et al. (15). Protein, 500 µg (100 µL), was mixed with 400 µL of 20-mM phosphate buffer (pH 7.4) and 500-µL reaction buffer (5-mg/mL thiobarburitic acid, 25-mM NaOH, and 50% glacial acetic acid). Samples were tightly sealed and boiled for 1 h and rested on ice for 10 min; the absorbance was measured at 532 nM. A standard curve (range 0–125 µmol/L) was used to determine MDA concentrations of samples.

Western blotting

Protein samples from cytosol and mitochondria were mixed with 5x loading buffer (30% glycerol, 10% sodium dodecyl sulfate [SDS], 0.31-M trisaminomethane (Tris) buffer, and 0.05% bromophenol blue). Samples were boiled for 3 min and optimal amounts (20 µg for 4-HNE detection, 6 µg for protein carbonyl detection, and 40 µg for mitochondrial cytochrome c detection) were loaded onto a 12% SDS-polyacrylamide gel electrophoresis (PAGE) and run at 120 V for 90 min. Equal loading was assessed with coommassie brilliant blue. Following electrophoresis, the gel was transferred onto a nitrocellulose membrane at 350 mA for 90 min. Ponceau S solution was used to assess equal band intensity and quality of transfer. The membrane was then incubated in blocking buffer (5% Marvel™ milk powder in 1x tween-tris buffered saline [TTBS[solution) for 1 h at room temperature and washed twice in 1X TTBS before overnight incubation with primary antibody dilutions (1:1000 for cytochrome c and 1:4000 for anti-dinitrophenyl [DNP] antibody). Following the incubation, the membrane was washed and incubated with secondary antibody (dilutions 1:20,000 for cytochrome c and 1:60,000 for anti-DNP) for 1 h at room temperature. The membrane was then incubated in West Pico ECL reagents (Thermofisher, Cramlington, UK) and exposed to Kodak photographic film (12). The resultant images were analysed using the Biorad GS-800 calibrated densitometer operating the Quantity One software.

Protein carbonyl formation

Protein oxidation was assessed by examining the formation of protein carbonyls using anti-DNP antibody. Proteincarbonylsform amino acids, especially lysine, arginine, threonine and proline when the protein is subjected to oxidation by free radicals. Samples were prepared according to the method described by Levine et al. (16). Protein sample, 15 µL, was mixed with 15 µL of 12% SDS solution and 30 µL of 1-mM 2,4-dinitrophenol hydrazine solution in 0.2-M HCl. After 15 min, samples were incubated at room temperature in the dark and 22.5 µL of neutralisation solution was added. Following the addition of 2-mercaptoethanol and loading buffer, the sample (unheated) was loaded on to a 12% SDS-PAGE gel and used as stated for the Western blotting procedure (as described above).

Caspase-3 activity

Caspase-3 activity was evaluated by measuring the fluorescence of N-acetyl-Asp-Glu-Val-Asp-AFC (7-amino 4-trifluoromethylcoumarin) (Ac-DEVD-AFC) after cleavage by caspase-3 to AFC following incubation with 25-µg protein. Samples were incubated in dark with 100-µL substrate solution (10 µg/mL Ac-DEVD-AFC, 100-mM HEPES, and 10-mM dithiothreitol [DTT]) for 1 h at 37°C.

The fluorescence was measured using an excitation wavelength of 380 nM and an emission wavelength of 520 nM. Caspase-3 activity was expressed as relative fluorescence units after correcting for substrate blank.

Antioxidant enzyme activity

The glutathione peroxidise cellular activity assay kit (Sigma, UK) was used as an indirect determination method based on the oxidation of GSH to glutathione disulfide (GSSG). Decrease in NADPH absorbance was measured at 340 nm, indicating GPx activity. GR assay kit (Sigma, UK) was used to measure the activity of GR, analysed by spectrophotometric measurement. The activity was measured by any increase in absorbance caused by the reduction of DTNB at 412 nm. The catalase assay kit (Abcam, UK) was used to measure catalase activity. Unconverted H_2O_2 reacts with OxiRedTM probe, which was analysed by spectrophotometric measurement at 570 nm.

Statistical analysis

Results were analysed using a one-way or two-way ANOVA withTukey's post-hoc analysis for multiple comparisons. Data were presented as mean ± SEM. P ≤ 0.05 was considered as statistically significant.

Results

Liver function

Following ethanol and cyanamide + ethanol treatment, ALT levels were significantly decreased by 72% and 83%, respectively, after 2.5 h (P < 0.0001). After 6 h, ethanol alone had

no effect on ALT levels; however, cyanamide with ethanol treatment resulted in a 74% reduction (P < 0.0001). At 24 h, ethanol and cyanamide + ethanol treatment caused a significant increase of 166% and 163%, respectively, in ALT levels (P < 0.0001). AST levels remained unchanged at both 2.5 and 6 h but increased by 80% (P < 0.05) following ethanol and cyanamide treatment. At 2.5 and 6 h, the AST/ALT levels were significantly increased (P < 0.0001) following cyanamide + ethanol treatment. Ethanol exposure alone had no effect on total protein or albumin levels after 2.5 h; however, cyanamide, followed by ethanol led to a 19% decrease in total protein (P < 0.0001) and a 17% decrease in albumin (P < 0.0001). After 6 h, ethanol caused a 12% reduction in total protein levels (P < 0.001) and a 14% decrease in albumin (P < 0.001) whereas cyanamide, followed by ethanol resulted in a 22% decrease in both total protein and albumin (P < 0.0001). At 24 h, total protein levels had recovered in ethanol alone-treated animals; however, albumin levels were still lower by 12% (P < 0.01). At 24 h, after cyanamide + ethanol treatment, albumin (35%; P < 0.0001) and total protein levels (29%; P < 0.0001) were still significantly impaired (Table 1).

Globulin levels remained unchanged by both ethanol only across all timepoints; however, cyanamide treatment + ethanol showed a significant decrease (P < 0.0001; Table 1).

Oxidative damage

After 2.5 h, ethanol alone led to a 28% decrease in cytosolic GSH whereas cyanamide with ethanol treatment resulted in a 31% decrease (P < 0.01; Figure 1A). At 6 h, ethanol alone led to a 16% reduction in cytosolic GSH with cyanamide with ethanol treatment showing a 23% reduction (P < 0.05). At 24 h, ethanol caused a reduction of 27% in GSH levels and cyanamide, with ethanol resulting in an 80% reduction (P < 0.0001; Figure 1A). In mitochondria, no significant changes were observed, although ethanol alone resulted in a 40% reduction after 2.5 h (Figure 1B). Cytosolic MDA levelswere increased by 69% (P < 0.0001) after 2.5 h of ethanol exposure; however, this increase was not observed with cyanamide + ethanol (Figure 1C). At 6 h, ethanol alone increased MDA levels by 32%, although there were no changes in other treatment groups. At 24 h, all treatment groups had

Table 1: Serum enzyme and protein levels following exposure to ethanol. Rats were pre-treated intraperitonially (i.p.) for 30 min with either saline (0.5 mol/L NaCl) or cyanamide (0.5 mmol/kg bodyweight), followed by injection of either saline (0.15 mol/L) or ethanol (75 mmol/kg bodyweight). Controls were injected with an identical volume of saline (0.5 mol/L NaCl). After 2.5, 6, and 24 h, the animals were sacrificed, and their blood was collected and analysed using standard biochemical methods.

Time	Treatment	ALT (U/L)	AST (U/L)	AST/ALT	Total Protein (g/L)	Albumin (g/L)	Globulin (g/L)
2.5 hr	Control	138 ± 11	321 ± 26	2.3 ± 0.1	46.8 ± 0.7	28.3 ± 0.4	18.5 ± 0.8
	Ethanol	38.5 ± 3.8****	154 ± 6.0	4.2 ± 0.5	44.3 ± 0.7	26.3 ± 0.3	18.0 ± 0.4
	Cyanamide	138 ± 7.2	316 ± 40	2.3 ± 0.2	48.0 ± 0.5	28.5 ± 0.4	19.5 ± 0.4
	Cyanamide/ Ethanol	24.4 ± 2.3****	249 ± 40	10.7 ± 1.7****	38.8 ± 0.7****	23.8 ± 0.7****	15.0 ± 0.3****
6 hr	Control	117 ± 3.7	268 ± 8.1	2.7 ± 0.2	48.8 ± 0.6	30.4 ± 0.4	18.4 ± 0.4
	Ethanol	114 ± 19	386 ± 77	3.3 ± 0.2	43.0 ± 0.7***	26.1 ± 0.6***	16.9 ± 0.3
	Cyanamide	116 ± 5.8	314 ± 18	2.7 ± 0.2	49.5 ± 0.9	29.8 ± 0.7	19.7 ± 0.8
	Cyanamide/ Ethanol	30.8 ± 3.9****	295 ± 69	9.9 ± 1.1****	38.7 ± 0.5****	23.9 ± 0.5****	14.8 ± 0.4****
24 hr	Control	56.2 ± 2.4	262 ± 39	4.7 ± 0.6	53.6 ± 0.7	30.4 ± 0.4	23.2 ± 0.9
	Ethanol	150 ± 20****	367 ± 38	3.0 ± 0.4	51.7 ± 1.7	26.7 ± 1.0**	25.0 ± 0.8
	Cyanamide	53.2 ± 6.2	262 ± 17	5.1 ± 0.4	52.7 ± 0.9	29.3 ± 0.6	23.3 ± 1.0
	Cyanamide/ Ethanol	140 ± 29****	470 ± 47*	3.7 ± 0.5	37.4 ± 1.3****	19.2 ± 1.6****	18.2 ± 0.5****

ALT: alanine aminotransferase; AST: aspartate aminotransferase. Values are expressed as mean ± SEM (n = 5–8). P ≤ 0.05. *P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001, ****P ≤ 0.0001 compared to corresponding controls.
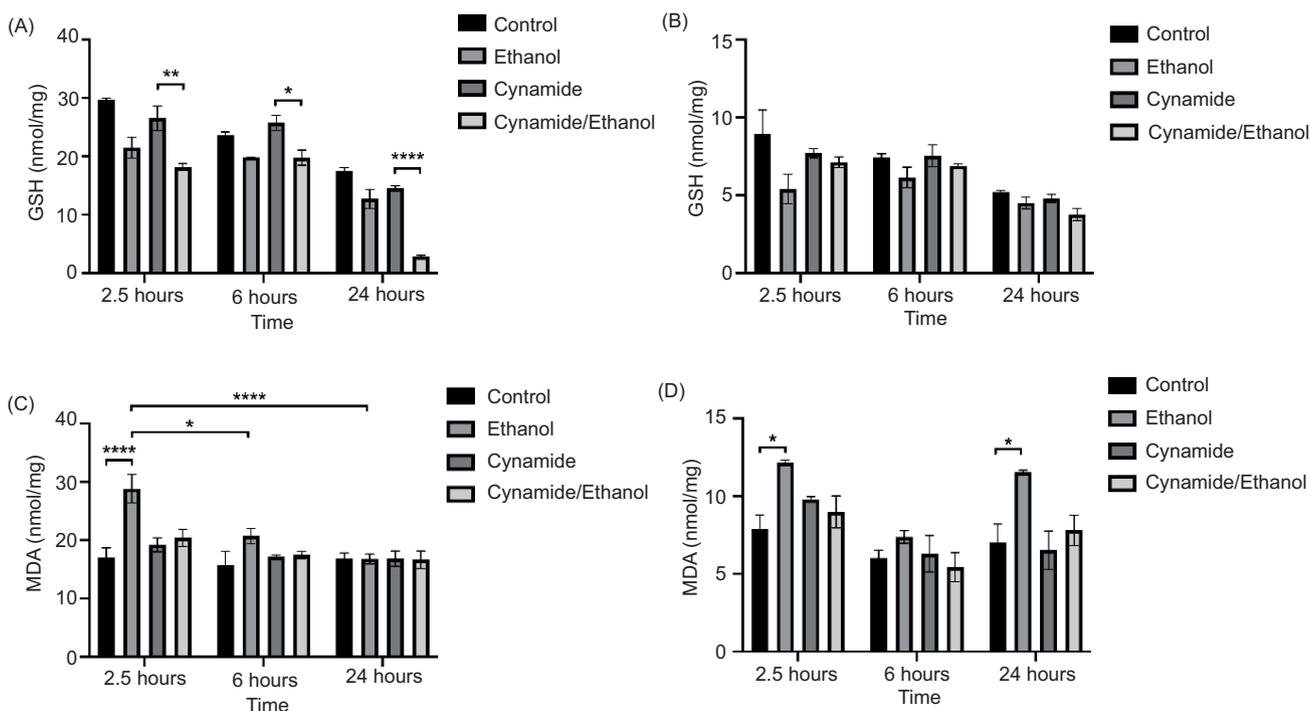
Figure 1: Effect of acute alcohol on hepatic GSH levels and MDA formation. (A) Cytosolic GSH levels, (B) mitochondrial GSH levels, (C) cytosolic MDA formation and (D) mitochondrial MDA formation. Following treatment, liver mitochondrial and cytosolic fractions were prepared as described previously. GSH levels were determined using 25 μg of protein, and MDA levels were determined by TBAR assay using 500 μg of protein. Values are expressed as mean ± SEM (n = 3–4). $^{*}P \leq 0.05$, $^{**}P \leq 0.01$, $^{****}P \leq 0.0001$ compared to other timepoints or relevant control within each timepoint. GSH: glutathione; MDA: malondialdehyde.

no effect on cytosolic MDA levels. Ethanol treatment led to an increase of 54% after 2.5 h in the levels of mitochondrial MDA (P < 0.05), a 44% increase after 6 h, and by 65% (P < 0.05) after 24 h (Figure 1D). No changes were observed with cyanamide + ethanol treatment. No significant changes were observed in cytosolic or mitochondrial protein carbonyl formation at any timepoint across all treatments (Figure 2).

Apoptosis

In the cytoplasm, ethanol caused a 70% rise in cytochrome c levels at 24 h (P < 0.05; Figures 3A–C). In the mitochondria, no significant changes were observed. At 24 h, ethanol treatment alone produced a 107% increase in caspase-3 activity (P < 0.001) and cyanamide + ethanol treatment resulted in a 51% increase in activity (Figure 3D).

Antioxidant enzyme activity

Following cyanamide + ethanol treatment, a 29% and 30% decrease was observed in cytosolic and mitochondrial GPx

activity, respectively (P < 0.01; Figures 4A and B). In the cytosol, cyanamide + ethanol produced a 30% increase in GR activity (P < 0.05; Figure 4C) whereas in the mitochondria, ethanol treatment alone led to a 94% increase (P < 0.05) in GR activity (Figure 4D). In the cytosol, treatment with ethanol alone and cyanamide + ethanol led to a 27% and 52% decrease, respectively, in superoxide dismutase (SOD) activity (P < 0.05; Figure 5A). In the mitochondria, ethanol only treatment led to a 30% reduction in SOD activity. Cyanamide + ethanol produced a 72% increase (P < 0.01) in SOD activity in the mitochondria (Figure 5B). The activity of catalase was also measured to assess its function. No changes in catalase activity were observed in either ethanol alone group or cyanamide + ethanol group (Figure 5C).

Discussion

Acute alcohol binge drinking is a major health issue (1,17). Alcohol has been shown to cause oxidative damage, increase ROS production as well as alter mitochondrial function and induce apoptosis (17). To date, limited studies have
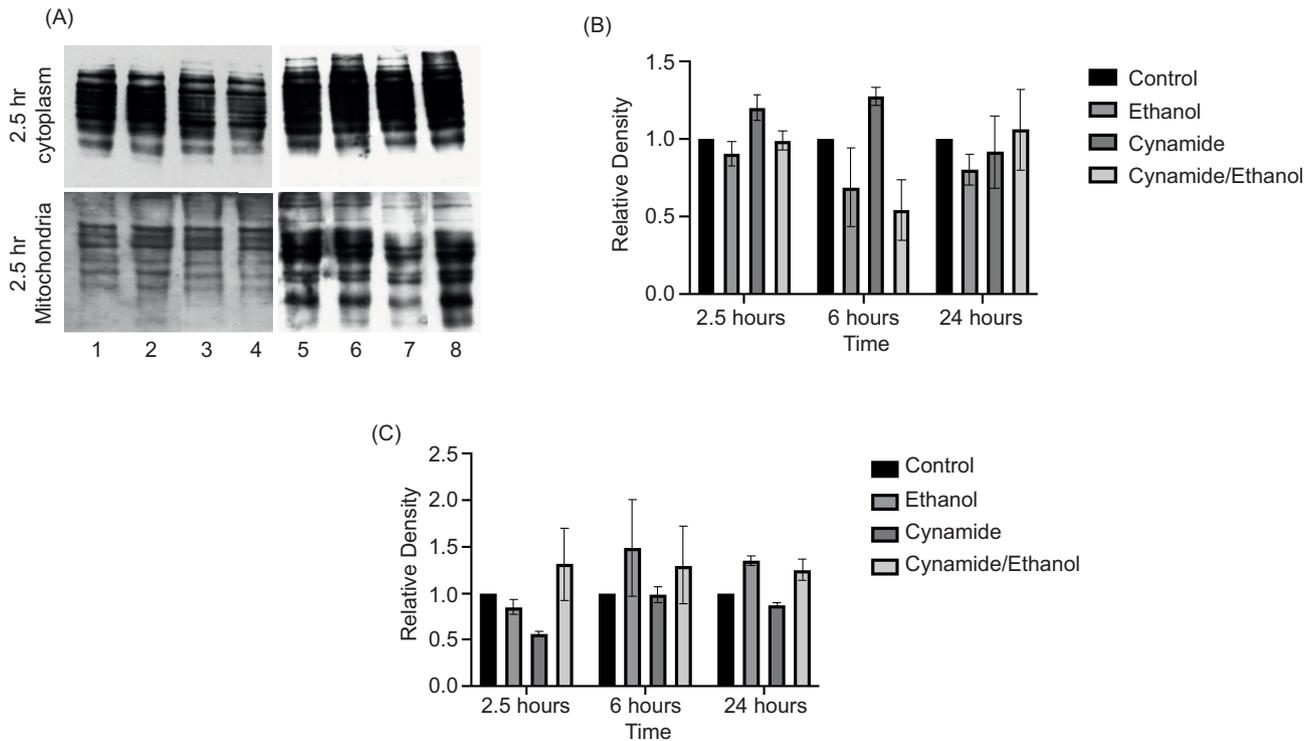
Figure 2: Effect of acute ethanol on protein carbonyl formation at 2.5, 6, and 24 h. (A) Representative immunoblot of protein carbonyl detection at 2.5 h, (B) cytosolic carbonyl protein levels and (C) mitochondrial carbonyl protein levels. Proteincarbonyls were detected by immunoblotting using an anti-DNP polyclonal primary antibody. Lanes 1 and 2: control, lanes 3 and 4: ethanol, lanes 5 and 6: cyanamide only, lanes 7 and 8: cyanamide + ethanol. Values are expressed as mean ± SEM (n = 3).

assessed the effects of both acute ethanol and acetaldehyde on oxidative damage and apoptosis in the liver over a 24-h period. This study aimed to investigate how acute ethanol and elevated acetaldehyde levels affected liver functioning by measuring changes in oxidative damage, apoptosis and antioxidant enzymes, primarily focussing on ethanol metabolism via the ADH pathway. Following ethanol exposure at 2.5 h, ALT levels were significantly decreased; however, after 6 h this had reversed in the ethanol-treated group but not in the cyanamide + ethanol group. The marked decrease has not been reported before as a direct effect of any treatment and warrants further investigation. This may indicate that hepatic functions have slowed as the liver attempts to metabolise ethanol, and/or is a result of low perfusion of the liver, since blood pressure is known to drop rapidly following an acute ethanol dose (18) and hypothermia arises by acute ethanol and acetaldehyde (19). This suggests that cyanamide pre-treatment and the resultant increase in acetaldehyde following ethanol metabolism is more harmful to the liver than ethanol alone. At 24 h, a more typical presentation of acute ethanol exposure was observed, supporting the findings from previous studies (20). Both ALT and AST

levels were increased; however, cyanamide and ethanol treatment enhanced the rise in AST. This may be an indicator of mitochondrial AST release, suggesting cell death, or damage to other cells, such as skeletal muscle (13) or erythrocytes, rather than hepatocytes alone. Acetaldehyde is known to bind to haemoglobin (21), perhaps causing haemolysis and thus releasing AST.

The liver is a major site of protein synthesis, and a decrease in serum protein levels generally indicates liver dysfunction. While ethanol reduced total protein and albumin, particularly at 6 h, cyanamide with ethanol treatment continuously reduced levels over the 24-h period, indicating significant hepatic impairment. This implicates acetaldehyde formation as a causative factor in reducing protein synthesis. While there are no studies examining the effect of acetaldehyde on liver protein synthesis, studies have shown in rat models that at 2.5 h, skeletal muscle (22) and jejunum synthesis rates are disturbed by up to 50% by cyanamide with ethanol treatment (23). Therefore, acetaldehyde may have a similar effect on protein synthesis in the liver. Ethanol exposure caused a decrease in GSH levels after 2.5 h, which generally continued to decrease over time. This
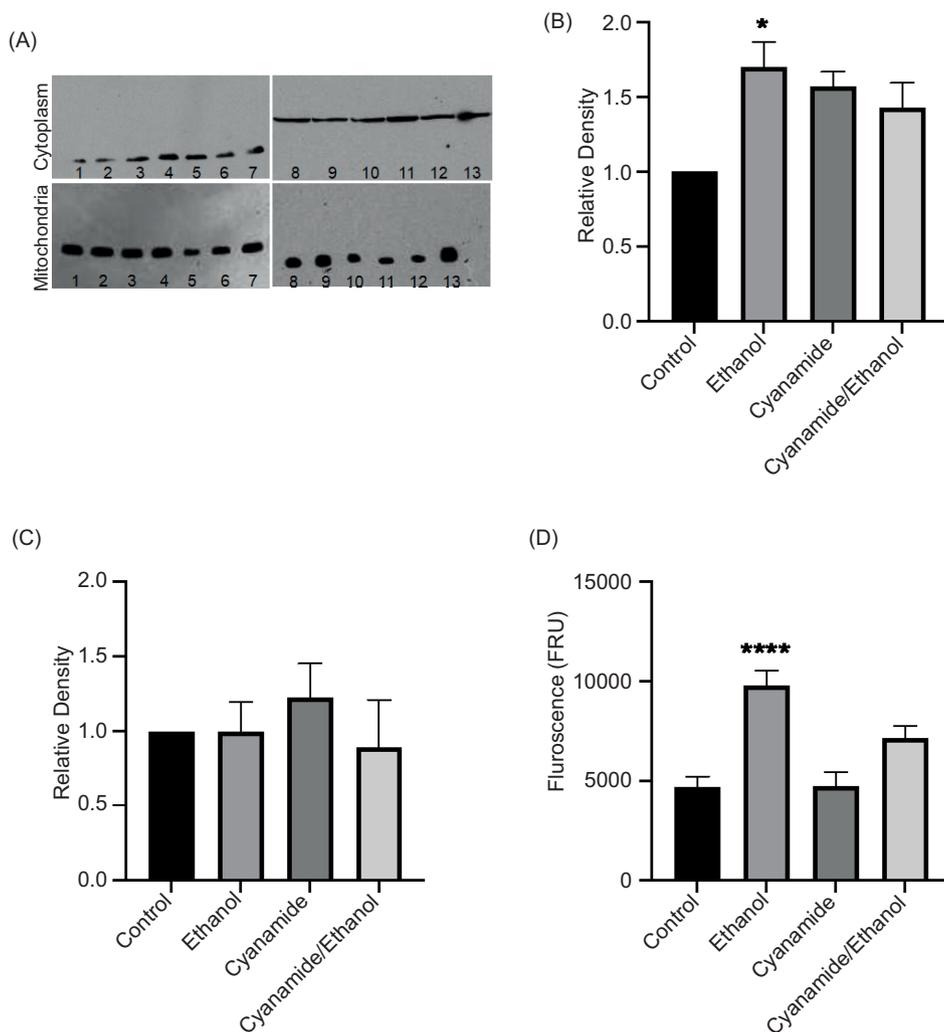
Figure 3: Effect of acute ethanol on cytochrome c levels at 24 h. (A) Cytochrome c immunoblot, (B) cytosolic cytochrome c levels, (C) mitochondrial cytochrome c levels and (D) cytosolic caspase-3 activity. Cytochrome c was detected by immunoblotting using a monoclonal cytochrome c antibody. Lanes 1–3: control; lanes 4–7: ethanol; lanes 8–10: cyanamide only and lanes 11–13: cyanamide and ethanol. Caspase-3 activity was determined using 25 µg of cytosolic protein by measuring the fluorescence of 7-amino-4-trifluoromethylcoumarin (AFC). Values are expressed as mean ± SEM (n = 3–4). $^*P \leq 0.05$, $^{****}P \leq 0.0001$, compared to relevant control.

pattern correlated with increased MDA levels, particularly at 2.5 and 6 h, which reflected results of previous studies (24). The mechanism of these changes is likely due to increased leakage of ROS from the mitochondrial electron transport chain (ETC) as a result of ethanol metabolism (25), causing GSH depletion and lipid peroxidation (1). The low GSH levels could be due to reduced production of adenosine 5'-triphosphate (ATP) as well as GSH binding to acetaldehyde for detoxification. Acetaldehyde can also directly damage isolated mitochondria, resulting in reduced mitochondrial respiration, lower ATP levels and decreased GSH in rat hepatocytes (26), with lower GSH caused by acetaldehyde impairing mitochondrial GSH uptake (8). This data indicate that the liver is under extreme stress, supporting earlier findings of compromised hepatic protein synthetic function due to acetaldehyde toxicity. Previous research has proposed that a decrease in mitochondrial GSH sensitises hepatocytes from ethanol-treated animals to tumour necrosis factor (TNF)-α-induced cell death (27). These results also suggest that cyanamide pre-treatment reduces detectable levels of oxidative damage caused by ethanol. Therefore, reducing the conversion of acetaldehyde to acetate with cyanamide may initially
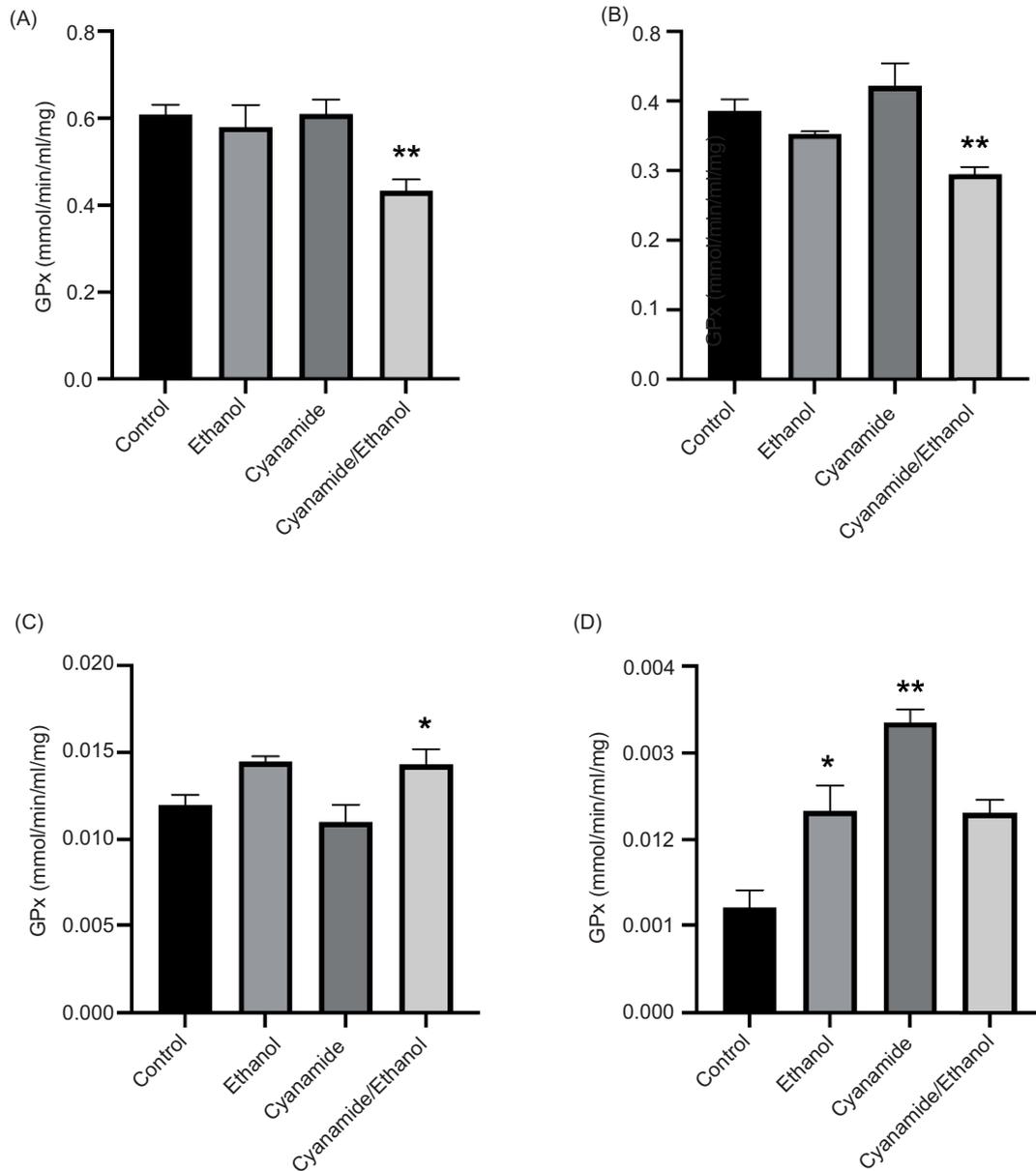
Figure 4: Effect of acute alcohol on hepatic antioxidant enzyme activity at 24 h. (A) Cytosolic GPx activity, (B) mitochondrial GPx activity, (C) cytosolic GR activity and (D) mitochondrial GR activity. Following treatment, liver mitochondrial and cytosolic fractions were prepared as described previously and the antioxidant enzyme activity was determined as described in the Methods section. Values are expressed as mean±SEM (n = 3–4). $^*P \leq 0.05$, $^{**}P \leq 0.01$, compared to control. GPx: glutathioneperoxidase; GR: glutathionereductase.

protect the liver, probably by reducing nicotinamide adenine dinucleotide hydrogen (NADH) formation and the rate of mitochondrial ROS production from the ETC. This theory is supported by studies showing that incubating rat hepatocytes with cyanamide and ethanol caused less ROS production than ethanol alone (25). Furthermore, in mice that lack aldehyde dehydrogenase 2 (*ALDH2*) gene, oxidative stress is lower when compared to wild-type ALDH2 mice as reflected by higher GSH levels at 6 h post-ethanol dose (28). Therefore, these findings show that the early effects of ethanol-induced oxidative stress are not fully mediated by acetaldehyde. No significant changes were observed in protein carbonylation.
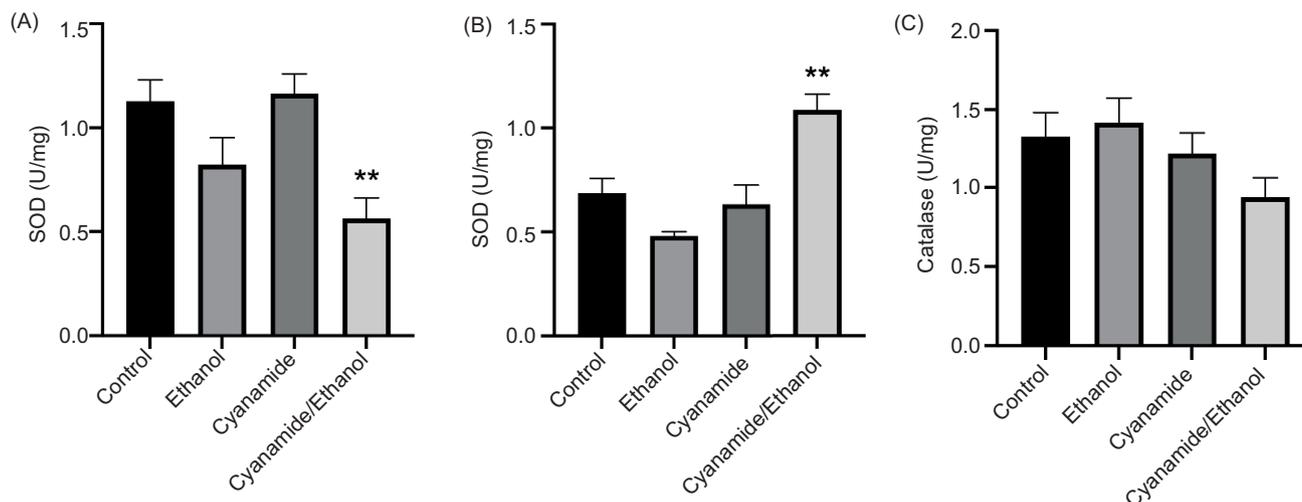
Figure 5: Effect of acute alcohol on hepatic antioxidant enzyme activity at 24 h. (A) Cytosolic SOD activity, (B) mitochondrial SOD activity and (C) catalase activity. Following the treatment, liver mitochondrial and cytosolic fractions were prepared as described previously and the antioxidant enzyme activity was determined as described in the Methods section. Values are expressed as mean ± SEM (n = 3–4). **$P \leq 0.01$, compared to control. SOD: superoxide dismutase.

However, increase in protein carbonyls does not necessarily reflect an outright increase in oxidative damage as acetaldehyde can bind to other proteins, including MDA and collagen forming protein carbonyls (29). The increase in cytochrome c observed in the cytoplasm at 24 h after ethanol alone treatment correlated with a significant increase in caspase-3 activity, suggesting the initiation of apoptosis, and is consistent with previous finding, where ethanol induces caspase3-dependent apoptosis (30). However, no differences were observed with cyanamide with ethanol treatment. This may be due to reduction in ATP production, and energetic failure may shut down apoptosis via oxidative stress-mediated caspase inactivation (31). Another possible explanation is the relationship between ATP, GSH and cytochrome c release. Results show a correlation between reduction in hepatic GSH levels and ATP production (32,33). Furthermore, prevention of GSH reduction also prevented reduction in ATP depletion (34), essentially proving a causal relationship. Previous studies have shown that chronic ethanol treatment lowers GPx activity in the cytoplasm and mitochondria (35), which has been supported by this study. Reduction in SOD activity may be due to ROS-induced enzyme degradation as well as reduced synthesis of enzymes due to acetaldehyde (36). This indicates ethanol and acetaldehyde-induced alterations in the antioxidant system, which may in turn promote oxidative damage in the liver. Previous research has shown that ethanol alters antioxidant system in a time-dependent manner (37). Decrease in GR as well as decrease seen in GSH levels indicates impairment in the regeneration of oxidised GSSG to reduced GSH, which occurs by the action of GR (37). The lower GPx activity suggests inactivation of enzymes as well as reduction of co-substrates such as GSH (38). We therefore speculate that the level of antioxidant enzyme activity post-ethanol treatment can differ due to the toxic effects of acetaldehyde (38).

Conclusion

Acute ethanol intake causes severe oxidative stress to the liver, which is initially mediated through NADH production via alcohol metabolism, promoting ROS leakage from the ETC. This was confirmed by cyanamide treatment which initially ameliorated the effect of alcohol, substantiating that oxidative damage is allied to NADH production. The liver initially undergoes enormous stress, as shown by reduced GSH. However, by 24 h the effects of acetaldehyde are particularly evident with depleted GSH and reduced GPx and SOD activity, compounding oxidative stress environment in the liver. In summary, this study has demonstrated that the liver is particularly susceptible to the toxic effects of elevated acetaldehyde levels.

Acknowledgments

Conflict of interest

None.

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