

Mitochondrial damage, cytotoxicity and apoptosis in iron-potentiated alcoholic liver fibrosis: amelioration by taurine

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Abstract Taurine effectively prevents ischemia-induced apoptosis in the cardiomyocytes and hypothalamic nuclei. The present study explores the influence of taurine on mitochondrial damage, oxidative stress and apoptosis in experimental liver fibrosis. Male albino Wistar rats were divided into six groups and maintained for a period of 60 days as follows: Group I, control; Group II, ethanol treatment [6 g/(kg/day)]; Group III, fibrosis induced by ethanol and iron (0.5% w/w); Group IV, ethanol + iron + taurine (2% w/v); Group V, ethanol + taurine treatment and Group VI, control + taurine treatment. Hepatocytes isolated from ethanol plus iron-treated rats showed decreased cell viability and redox ratio, increased reactive oxygen species formation, lipid peroxidation, DNA fragmentation, and formation of apoptotic bodies. Liver mitochondria showed increased susceptibility to swell, diminished activities of mitochondrial respiratory chain complexes and antioxidants. Taurine administration to fibrotic rats restored mitochondrial function, reduced reactive oxygen species formation, prevented DNA damage, and apoptosis. Thus taurine might contribute to the amelioration of the disease process.

Keywords Ethanol · Carbonyl iron · Taurine · Cytotoxicity · DNA damage · Mitochondria · Fibrosis · Apoptosis

Introduction

Chronic ethanol administration in experimental animals significantly increases the percentage of apoptotic hepatocytes (Yacoub et al. 1995). A link between ethanol metabolism, cytochrome P450 isoenzyme (CYP2E1) induction, enhanced reactive oxygen species (ROS) formation, lipid peroxidation, and cytotoxicity of apoptotic nature has been actively studied and documented (Lieber 1997). Impairment of mitochondrial function is a prelude to the apoptotic event. Mitochondrial abnormalities induced by ethanol are disruption of electron transport chain, oxidative phosphorylation, and ATP generation, all of which are believed to result from increased ROS production (Green and Reed 1998). García-Ruiz et al. (1994) observed loss of functional integrity of mitochondria as a cause of increased ROS production which contributes to apoptotic death. Reduced glutathione (GSH) is the most abundant non-protein antioxidant in the cells which plays a pivotal role in the defense against oxidative stress-induced cell injury and mitochondrial damage (Dickinson and Forman 2002). The concentrations of cellular and mitochondrial GSH are reported to be low in ethanol-fed animals (Fernandez-Checa and Kaplowitz 2005).

Taurine (2-aminoethane sulfonic acid) is a conditionally essential, non-protein amino acid present in blood and tissues of mammals. It plays many important roles in the body-like bile acid conjugation, detoxification, membrane stabilization, osmoregulation, calcium homeostasis, and neurotransmission (Huxtable 1992). Many studies suggest that taurine supplementation can lower oxidative stress (Balkan et al. 2001; Schaffer et al. 2003). The liver-protective role of taurine against toxic insult has been shown (Hwang and Wang 2001; Tabassum et al. 2006). In our earlier study, we observed that taurine circumvents

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oxidative stress and liver pathology in experimental alcoholic liver disease (Pushpakiran et al. 2004, 2005). Taurine has also been reported to have anti-apoptotic properties (Wu et al. 1999; Takatani et al. 2004).

The spectrum of alcoholic liver disease ranges from minimal injury to hepatitis, fibrosis, and cirrhosis. However, studies have observed that rats fed alcohol alone do not develop alcoholic liver disease beyond the stage of fatty liver, the addition of iron exacerbates the oxidative stress induced by alcohol, and rats treated with a combination of iron and alcohol develop liver damage faster than rats given alcohol alone (Caro and Cederbaum 2001). Thus the combination of iron and alcohol as a model of liver fibrosis was chosen. The study investigates whether taurine could ameliorate mitochondrial dysfunction, DNA damage, and apoptosis in liver of fibrotic rats.

Materials and methods

Chemicals

Iron carbonyl, rotenone, coenzyme Q1 and Q2, antimycin A, dodecyl- β -D-maltoside cytochrome *c*, propidium iodide, RNAase A, and ethidium bromide were purchased from Sigma Aldrich Pvt. Ltd., St. Louis, MO, USA. Taurine was obtained from Sisco Research Laboratories Pvt. Ltd., Mumbai, India. The solvents and other chemicals used were of analytical grade and purchased from Himedia Laboratories Pvt. Ltd., Mumbai, India.

Treatment of animals

Male albino rats of Wistar strain weighing 170–190 g were purchased from the Central Animal House, Rajah Muthiah Medical College, Annamalai University. The animals were housed in polypropylene cages and maintained under controlled conditions of 12 h light/12 h dark cycle and 50% humidity at 25–30°C. The animals were fed pellet diet (Kamadhenu Agencies, Bangalore, India) and water ad libitum. The study was approved by Institutional Animal Ethics Committee (IAEC), Rajah Muthiah Medical College and Hospital, Annamalai University, and the animals were maintained in accordance with the Guide for the Care and Use of Laboratory Animals. After a period of 1 week, the animals were divided into 6 groups of 12 rats each.

Group I received glucose (5 g/kg) isocaloric to ethanol from 40% glucose stock solution.

Group II received ethanol at a dosage of 3 g/kg from 30% stock solution twice daily [6 g/(kg/day)].

Group III received ethanol and iron carbonyl (0.5% w/w).

Group IV received ethanol, iron, and taurine (2% w/v).

Group V received ethanol and taurine.

Group VI received isocaloric glucose and taurine.

Ethanol and glucose were administered by oral gavage. Iron carbonyl was added to the powdered diet at the level of 0.5% w/w (Bacon et al. 1983). Taurine was administered in drinking water (2% w/v) (Anitha Nandhini et al. 2002). Food and water were fed ad libitum to all groups.

At the end of the experimental period (60 days), six rats from each group were fasted overnight, administered ketamine hydrochloride (30 mg/kg bw) and killed. The animals were cut opened, liver was dissected out and washed in ice-cold saline, blotted, weighed, and mitochondria were isolated for further studies.

Preparation of mitochondria, and measurement of electron transport chain activity and mitochondrial swelling

Mitochondria were isolated by differential centrifugation (Uysal et al. 1989). The protein content was measured by the method of Lowry et al. (1951) and purity was checked by assaying succinate dehydrogenase activity (Slater and Bonner 1952). The electron transport chain activity was determined by measuring the mitochondrial activities of complexes I, II, III, and IV. Mitochondrial samples were subjected to three cycles of fast freeze–thaw in hypotonic buffer (20 mM potassium buffer, pH 7.2) prior to the assay. The activities of complexes I, II, and III were assayed as described by Brich-Machin et al. (1993) with a few modifications. The assay mixture for complex I contained 25 mM potassium phosphate buffer, pH 7.2, 5 mM MgCl_2 , 2 mM potassium cyanide (KCN), 2.5 mg/ml bovine serum albumin, 0.13 mM NADH, 65 μM coenzyme Q1, and 2 $\mu\text{g}/\text{ml}$ antimycin A. Mitochondrial sample was added to initiate the reaction and the oxidation was monitored at 340 nm (ϵ 6.81 $\text{mM}^{-1} \text{cm}^{-1}$) for 1 min. For complex II (succinate dehydrogenase) assay, mitochondrial sample was preincubated in a medium containing 25 mM potassium phosphate buffer, pH 7.2, 5 mM MgCl_2 , and 20 mM succinate for 10 min at 30°C. Antimycin A (2 $\mu\text{g}/\text{ml}$), rotenone (2 $\mu\text{g}/\text{ml}$), KCN (2 mM), and 2, 6-dichlorophenol indophenol (50 mM) were added and further incubated for 1 min. The reaction was initiated with coenzyme Q1 (65 μM) and the enzyme-catalyzed reduction of 2, 6-dichlorophenol indophenol was recorded for 3 min at 600 nm (ϵ 19.1 $\text{mM}^{-1} \text{cm}^{-1}$). The activity of complex III (coenzyme Q cytochrome *c* reductase) was determined by measuring the rate of reduction of cytochrome *c* (III) by the reduced form of coenzyme Q2 (ubiquinol) at 550 nm (ϵ 20 $\text{mM}^{-1} \text{cm}^{-1}$). Complex IV (cytochrome *c* oxidase) assay was carried out by

the method of Wharton and Tzagoloff (1967). The measurement is based on the decrease in absorbance at 550 nm by the oxidation of ferrocytochrome by cytochrome *c* oxidase.

For swelling studies, the medium contained mitochondrial pellet (0.5 mg protein ml⁻¹), 125 mM sucrose, 10 mM HEPES buffer, pH 7.2, 2.5 mM succinate, and 4.0 μM rotenone in a final volume of 1.0 ml. Mitochondrial swelling was evaluated by monitoring the change in absorbance at 520 nm in a spectrophotometer for 5 min. Decreasing values of absorbance indicate mitochondrial swelling (Garlid and Beavis 1985).

Antioxidant enzymes and reduced glutathione status

The activity of superoxide dismutase (SOD, EC.1.15.1.1), catalase (CAT, EC.1.11.1.6), and glutathione peroxidase (GPx, (EC.1.15.1.9) were measured in mitochondria by methods described in detail elsewhere (Anitha Nandhini et al. 2002). Glutathione reductase (GR) was assayed by the method of Horn and Burn (1978). The GSH content in mitochondria was determined according to the method of Tietze (1969).

Isolation of hepatocytes

Hepatocytes were isolated from a set of six rats in each group. Rats were fasted for 24 h and anesthetized with ketamine hydrochloride (30 mg/kg bw, ip), and the hepatocytes were isolated by the collagenase perfusion technique (Seglen 1976) and used for the following assays.

Cell viability and MTT assay

Cell viability was measured by the ability of the living cells to exclude trypan blue vital dye. Cells were washed twice with phosphate-buffered saline (PBS) and trypan blue in PBS was added at a final concentration of 0.2%. Living cells that did not take up the stain were counted in a hemocytometer and expressed as the percentage of the total count. Mitochondrial function was assayed by the following conversion of MTT 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide) by mitochondrial dehydrogenases to purple formazan crystals. Factors that inhibit dehydrogenase activity will affect the associated color formation. Hepatocytes were incubated in the dark at 37°C with 50 μl MTT (1 mg/ml) for 4 h. After incubation, the medium was removed, the formazan crystals were dissolved in 200 μl of dimethylsulfoxide (DMSO), and the absorbance was measured at 570 nm. The percentage reaction was calculated assuming 100% reaction for hepatocytes from control rats.

Reactive oxygen species formation, lipid peroxidation and redox status

Intracellular superoxide formation was determined according to the method of Eligini et al. (2005). An aliquot of hepatocyte (0.1 ml) was incubated with 0.5 ml of nitroblue tetrazolium (NBT) (final concentration 1 mg ml⁻¹) for 30 min. The cells were then treated with 0.6 ml of a solution containing 90% DMSO, 0.01 N NaOH, and 0.1% sodium dodecyl sulfate (SDS). Absorbance was measured at 630 nm. NBT reduction correlates with the amount of superoxide (O²⁻) produced by the cells. Cellular lipid peroxidation was determined by assaying the formation of thiobarbituric acid substance (TBARS) by spectrophotometry (Niehaus and Samuelsson 1968). Briefly, a 0.5 ml aliquot of the cell suspension was added to 2.0 ml a reagent containing trichloroacetic acid:thiobarbituric acid:hydrochloric acid reagent (TCA:TBA:HCl in 1:1:1 ratio). The reaction mixture was heated in a boiling water bath for color development, cooled to room temperature, and centrifuged to remove insoluble materials. The absorbance of clear supernatant was measured at 535 nm. Lipid hydroperoxide (LHP) was determined according to the method of Jiang et al. (1992). An aliquot of hepatocyte (0.1 ml) was incubated with 0.9 ml of FOX reagent for 30 min at room temperature and the absorbance was measured at 560 nm. Reduced and oxidized forms of glutathione (GSH and GSSG) in hepatocytes were estimated by the method of Tietze (1969) and expressed as a ratio (GSH/GSSH). Protein content of the cells was measured by the method of Lowry et al. (1951).

Propidium iodide (PI) staining

Hepatocytes were washed in PBS thrice and incubated for 10 min with 50 μl of PI (5 mg/ml), mounted in slides, and examined by fluorescence microscopy (Olympus BX51).

DNA fragmentation assay

The DNA fragmentation pattern was analyzed by agarose gel electrophoresis. Hepatocytes were collected and centrifuged at 1,000×*g* for 10 min. The cell pellets were suspended in 1 ml of lysis buffer consisting of 50 mM Tris-HCl, pH 8.0, 10 mM NaCl, 10 mM EDTA, 100 μg/ml proteinase K, and 0.5% SDS and incubated for 1 h at 50°C. 10 μl of 100 μg/ml ribonuclease A (RNase A) was added to the mixture and incubated for an additional 1 h at 50°C. DNA samples were treated with 1 ml phenol followed by extraction with chloroform/isoamylalcohol. The aqueous phase was treated with 25–50 μl of 3 M sodium acetate (pH 5.2) and one volume of ethanol, shaken gently, and left at -20°C, overnight. The precipitate was collected by

centrifugation at $12,000\times g$ for 20 min. The pellets were rinsed with 1 ml of 70% ethanol and spun for 10 min. The supernatant was discarded and the pellet was air dried at room temperature and later dissolved in 0.5–1.0 ml of double distilled water. DNA fragmentation was assayed by electrophoresing in 1.0% agarose/ethidium bromide gel for 90 min at 90 V and photographed with UV transilluminator.

Statistical analysis

Results are presented as mean $\pm$ SD. Data were analyzed by one-way analysis of variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT) using the statistical software package (SPSS for Windows, V. 13.0, Chicago, IL, USA).

Results

The contents of TBARS, LHP, GSH, GSSG, and redox ratio in rat hepatocytes are given in Table 1. An increase in TBARS and LHP was found in ethanol and ethanol plus iron-treated animals. The increase was more pronounced in hepatocytes isolated from ethanol plus iron-treated rat hepatocytes. Supplementation with taurine lowered the levels of TBARS and LHP. The redox ratio was significantly lower in ethanol and ethanol plus iron-treated rats as compared to control hepatocytes. Treatment with taurine restored the ratio to near control values.

Viability of cells from each group is expressed as percentage of total cells. There was a decrease (47%) in cell viability in ethanol-treated rats and 36% in ethanol plus iron-treated rats (Fig. 1). Taurine treatment prevented cell

death induced by ethanol and ethanol plus iron as indicated by the increased cell viability of about 74 and 66%, respectively. Figure 2 illustrates the percentage of MTT metabolism in hepatocytes of animals from various groups. Hepatocytes isolated from ethanol-treated rat showed decreased MTT metabolism (45%) while ethanol plus iron-treated rats showed a further decrease in MTT metabolism (36%). In the presence of taurine, MTT metabolism was increased to 78 and 67% in ethanol and ethanol plus iron treated rats, respectively.

Intracellular superoxide levels in the hepatocytes obtained from ethanol and ethanol plus iron treated groups compared to those from control animals were significantly higher (Control $13.39 \pm 1.02 \mu\text{mol ml}^{-1}$; ethanol $23.94 \pm 1.82 \mu\text{mol ml}^{-1}$; ethanol + iron $32.29 \pm 2.46 \mu\text{mol ml}^{-1}$). Taurine treatment significantly decreased the level of superoxide by its antioxidant property (ethanol + iron + taurine $20.78 \pm 1.58 \mu\text{mol ml}^{-1}$; ethanol + taurine $15.05 \pm 1.14 \mu\text{mol ml}^{-1}$).

Table 2 represents the activities of respiratory chain complexes in liver mitochondria from experimental animals. Statistically significant reduction in the activities of respiratory chain complexes was observed in ethanol, ethanol plus iron-treated rats. Administration of taurine significantly ($P < 0.05$) increases the values to near-normal.

Table 3 gives the activities of the antioxidant enzymes and glutathione levels in various experimental groups. The enzymes SOD, CAT, GPx, and GR in ethanol plus iron-treated groups showed decreased activity by 35, 49, 40, and 41%, respectively, and GSH by 32% compared to control rats. Taurine administration significantly increased the activities of antioxidant enzymes and GSH levels.

Table 1 Protective effect of taurine on ethanol and iron-induced lipid peroxidation and cellular redox state in hepatocyte of experimental animals

Parameters	CON	EtOH	EtOH + Fe	EtOH + Fe + TAU	EtOH + TAU	CON + TAU
TBARS ^a	1.31 ± 0.11	$6.23 \pm 0.47^*$	$8.82 \pm 0.67^{* \dagger \ddagger}$	$5.64 \pm 0.43^\ddagger$	$3.42 \pm 0.26^\ddagger$	1.30 ± 0.11
LHP ^b	4.87 ± 0.37	$8.34 \pm 0.64^*$	$12.54 \pm 0.95^{* \dagger \ddagger}$	$10.12 \pm 0.77^\ddagger$	$6.23 \pm 0.47^\ddagger$	4.94 ± 0.38
GSH ^b	53.24 ± 4.05	$32.49 \pm 2.47^*$	$25.41 \pm 1.92^{* \dagger \ddagger}$	$38.24 \pm 2.91^\ddagger$	$45.13 \pm 3.44^\ddagger$	54.35 ± 4.14
GSSG ^b	3.82 ± 0.29	$10.24 \pm 0.78^*$	$13.12 \pm 0.99^{* \dagger \ddagger}$	$9.34 \pm 0.71^\ddagger$	$5.31 \pm 0.40^\ddagger$	3.89 ± 0.29
GSH/GSSG ratio	13.94 ± 1.06	$3.17 \pm 0.24^*$	$1.85 \pm 0.14^{* \dagger \ddagger}$	$4.09 \pm 0.31s^\ddagger$	$8.50 \pm 0.65^\ddagger$	14.01 ± 1.06

Values are means $\pm$ SD of six rats from each group

CON control, EtOH ethanol, EtOH + Fe ethanol + iron, EtOH + Fe + TAU ethanol + iron + taurine, EtOH + TAU ethanol + taurine
CON + TAU control + taurine

* Significant as compared to control ($P < 0.05$, ANOVA followed by DMRT)

[†] Significant as compared to ethanol ($P < 0.05$, ANOVA followed by DMRT)

[‡] Significant as compared to ethanol + iron ($P < 0.05$, ANOVA followed by DMRT)

^a nmol/g wet tissue

^b nmol/mg protein

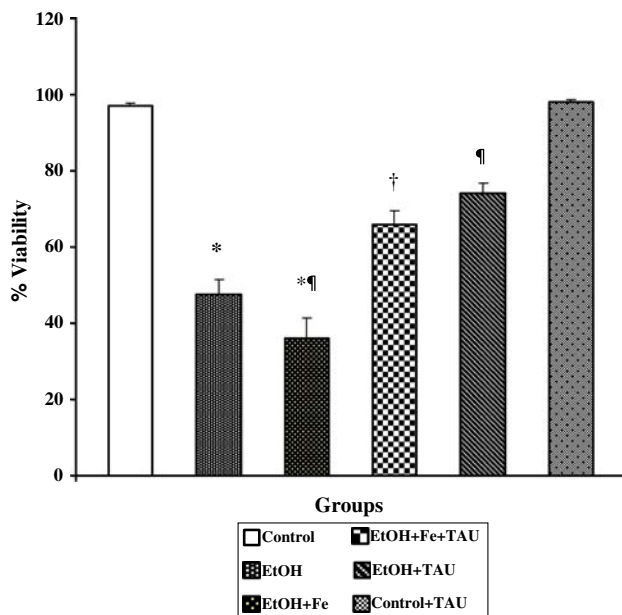


Fig. 1 Viability of hepatocytes expressed as percentage (%) of control. Values are means $\pm$ SD ($n = 6$ in each group). Analysis of variance followed by DMRT, $P < 0.05$. *Significant as compared to control, †significant as compared to ethanol, ‡significant as compared to ethanol + iron. CON control, EtOH ethanol, EtOH + Fe ethanol + iron, EtOH + Fe + TAU ethanol + iron + taurine, EtOH + TAU ethanol + taurine, CON + TAU control + taurine

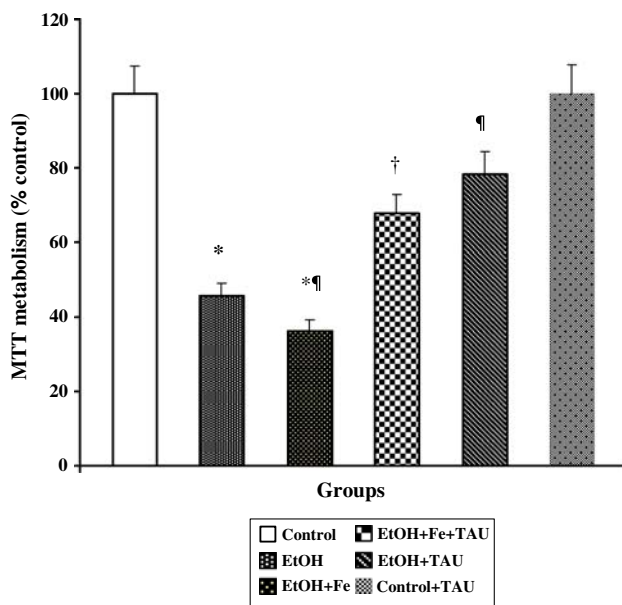


Fig. 2 Effect of taurine on MTT metabolism of experimental groups. Values are means $\pm$ SD ($n = 6$ in each group). Analysis of variance followed by DMRT; $P < 0.05$. *Significant as compared to control, †significant as compared to ethanol, ‡significant as compared to ethanol + iron. CON control, EtOH ethanol, EtOH + Fe ethanol + iron, EtOH + Fe + TAU ethanol + iron + taurine, EtOH + TAU ethanol + taurine, CON + TAU control + taurine

The ability of mitochondria to swell under experimental conditions is a technique that is easy to perform, and is considered to be a sensitive and reliable technique to study the prooxidant-induced permeability of mitochondria. Figure 3 shows the susceptibility of mitochondria to swell. Mitochondria from ethanol group and ethanol plus iron-treated group showed increased swelling as compared to those from control group. The swelling was significantly blunted in TAU-treated group indicating that TAU is able to protect the mitochondrial membrane.

Figure 4a–f shows hepatocyte morphology after PI staining. Cells from control animals exhibited healthy nuclei (Fig. 4a, f), while hepatocytes isolated from ethanol, ethanol plus iron treated groups showed highly fluorescent, condensed, and fragmented chromatin suggesting apoptosis mode of cell death (Fig. 4b, c). Treatment with taurine showed regular morphology with round contours and reduced uptake of the dye (Fig. 4d, e).

The extent of DNA damage in rat hepatocytes was determined by DNA fragmentation assay. The results are shown in Fig. 5. An increased DNA damage and streak formation were observed in ethanol plus iron-treated rats (lane 3) as compared to normal animals (lane 2). Ethanol-treated rats showed decreased DNA damage (lane 4) when compared to ethanol plus iron-treated rats (lane 3). No DNA damage was observed in control (lane 2) and taurine-treated animals (lanes 5–7).

Discussion

Studies have confirmed that the toxicity of ethanol in the liver is due to generation of ROS, depletion of GSH, increased lipid peroxidation, mitochondrial injury, and DNA modification in the liver, where it is oxidized (Nordmann et al. 1992). In our study addition of iron to ethanol-fed rats resulted in marked toxicity. Iron can propagate lipid peroxidation through ferryl–perferryl type oxidants and diverse lipid-derived alkoxyl or peroxy radicals (Caro and Cederbaum 2001). Addition of iron and ethanol to HepG2 cell lines that overexpress CYP2E1 decreases cell viability and causes apoptosis (Wu and Cederbaum 1996). Iron accumulation is another potential cause of enhanced ROS formation in alcoholic liver disease in humans and associated with increased apoptosis of hepatocytes (Kato et al. 1996). Several investigators have used this combination of treatment at varying concentrations (Stal and Hultcrantz 1993; Tsukamoto et al. 1995; Valerio et al. 1996) to produce fibrosis. The primary reason for the dietary supplementation of iron in addition to intragastric infusion of ethanol was to increase the hepatic non-heme iron higher than normal rather than to produce iron overload. We

Table 2 Effect of taurine on liver mitochondrial respiratory chain complexes of experimental animals

Parameters	CON	EtOH	EtOH + Fe	EtOH + Fe + TAU	EtOH + TAU	CON + TAU
Complex I ^a	1.36 ± 0.12	0.88 ± 0.074*	0.71 ± 0.061* [¶]	0.99 ± 0.085 [†]	1.16 ± 0.10 [¶]	1.37 ± 0.10
Complex II ^b	1.52 ± 0.12	1.13 ± 0.92*	0.94 ± 0.84* [¶]	0.79 ± 0.63 [†]	0.67 ± 0.53 [¶]	1.53 ± 0.12
Complex III ^c	1.72 ± 0.13	1.32 ± 0.10*	0.99 ± 0.082* [¶]	1.18 ± 0.10 [†]	1.52 ± 0.12 [¶]	1.74 ± 0.13
Complex IV ^d	2.62 ± 0.20	1.97 ± 0.15*	1.52 ± 0.12* [¶]	1.76 ± 0.13 [†]	2.35 ± 0.18 [¶]	2.64 ± 0.20

Values are means ± SD of six rats from each group

CON control, EtOH ethanol, EtOH + Fe ethanol + iron, EtOH + Fe + TAU ethanol + iron + taurine, EtOH + TAU ethanol + taurine
CON + TAU control + taurine

* Significant as compared to control ($P < 0.05$, ANOVA followed by DMRT)

[¶] Significant as compared to ethanol ($P < 0.05$, ANOVA followed by DMRT)

[†] Significant as compared to ethanol + iron ($P < 0.05$, ANOVA followed by DMRT)

^a μmol of NADH oxidized/min/mg protein

^b μmol of DCIP reduced/min/mg protein

^c μmol of cytochrome *c* reduced/min/mg protein

^d μmol of cytochrome *c* oxidized/min/mg protein

Table 3 Protective effect of taurine on mitochondrial antioxidants and GSH of experimental animals

Parameters	CON	EtOH	EtOH + Fe	EtOH + Fe + TAU	EtOH + TAU	CON + TAU
SOD ^a	28.13 ± 2.14	15.34 ± 1.17*	10.03 ± 0.76* [¶]	19.23 ± 1.46 [†]	22.97 ± 1.75 [¶]	28.13 ± 2.19
CAT ^b	35.13 ± 2.67	22.36 ± 2.74*	17.23 ± 1.31* [¶]	28.88 ± 2.19 [†]	31.33 ± 2.39 [¶]	35.99 ± 2.74
GPx ^c	6.92 ± 0.52	4.23 ± 0.32*	2.78 ± 0.21* [¶]	5.02 ± 0.38 [†]	6.12 ± 0.47 [¶]	6.94 ± 0.53
GR ^d	12.84 ± 0.98	7.45 ± 0.57*	5.43 ± 0.41* [¶]	9.68 ± 0.73 [†]	11.32 ± 0.84 [¶]	12.93 ± 0.98
GSH ^e	51.55 ± 3.92	26.13 ± 1.99*	16.56 ± 1.26* [¶]	32.35 ± 2.46 [†]	41.35 ± 3.15 [¶]	52.24 ± 3.98

Values are means ± SD of six rats from each group

CON control; EtOH ethanol; EtOH + Fe ethanol + iron; EtOH + Fe + TAU ethanol + iron + taurine; EtOH + TAU ethanol + taurine
CON + TAU control + taurine

* Significant as compared to control ($P < 0.05$, ANOVA followed by DMRT)

[¶] Significant as compared to ethanol ($P < 0.05$, ANOVA followed by DMRT)

[†] Significant as compared to ethanol + iron ($P < 0.05$, ANOVA followed by DMRT)

^a units 50% of NBT reduction/min/mg protein

^b μmol of H₂O₂ consumed/min/mg protein

^c μmol of GSH oxidized/min/mg protein

^d μmol of NADPH oxidized/h/mg protein

^e nmol/mg protein

observed accumulation of iron in both ethanol alone and ethanol plus iron-treated rats.

The ability of ethanol to stimulate mitochondrial ROS production in the hepatocytes is linked to the ability of ethanol to damage the oxidative phosphorylation system (Bailey and Cunningham 2002). Studies have conclusively shown that ethanol alters the concentrations and/or functions of respiratory chain complexes (Hoek 1994). Chronic ethanol consumption decreases the content of iron–sulfur clusters of complex I (Thayer et al. 1980) and levels of cytochrome *b* in complex III (Cunningham et al. 1990). Chen et al. (2000) observed that liver cytochrome *c* oxidase is susceptible to oxidative modification and

inactivation by the lipid peroxidation products. Prolonged ethanol treatment has been shown to decrease the activity and heme content of cytochrome *c* oxidase (Spach et al. 1982). Damage to respiratory chain complexes decreases the rate of ATP synthesis which is likely to contribute to the damage observed in hepatocytes. Mitochondrial swelling studies reveal damage to the organelle in ethanol and ethanol plus iron-treated rats. Oxidative stress, membrane damage, and increased membrane pore transition (MPT) are suggested to influence mitochondrial swelling (Cao et al. 2004). Defects in osmoregulation and disturbances in Ca²⁺ modulation also contribute to mitochondrial swelling.

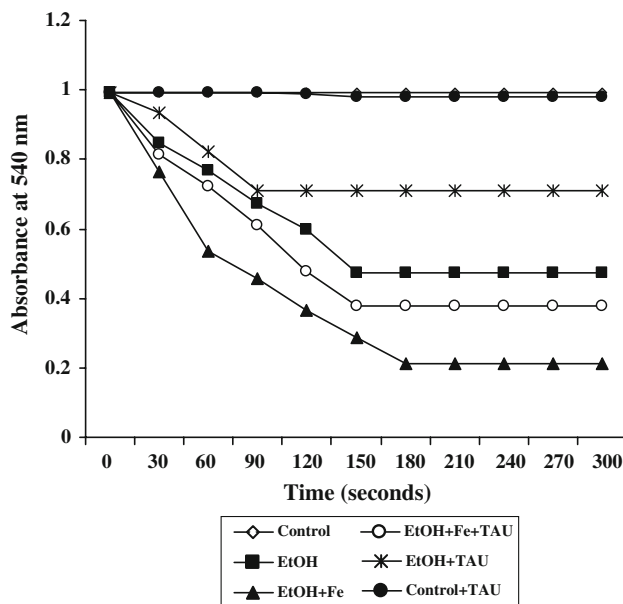


Fig. 3 Mitochondrial swelling in ethanol, ethanol plus iron and the effect of taurine. Values are means $\pm$ SD ($n = 6$ in each group). Analysis of variance followed by DMRT, $P < 0.05$. *Significant as compared to control, [†]significant as compared to ethanol, [‡]significant as compared to ethanol + iron. CON control; EtOH ethanol; EtOH + Fe ethanol + iron; EtOH + Fe + TAU ethanol + iron + taurine; EtOH + TAU ethanol + taurine; CON + TAU control + taurine

Chronic exposure to ethanol may alter GSH homeostasis by decreasing the activity of the GSH regulating enzyme, GR (Polavarapu et al. 1998) or by inhibition of synthesis or transport (Fernández-Checa et al. 1998). Also, acetaldehyde, the product of ethanol oxidation can bind to GSH and cause depletion of GSH.

Induction of CYP2E1 by ethanol predisposes to the MPT via ROS production (Cederbaum 2001). The MPT is the regulatable opening of a large, non-specific pore across the outer and inner mitochondrial membrane. Mitochondrial depolarization is followed by cytochrome *c* release from the mitochondria into cytosol, caspase activation, and apoptosis (Higuchi et al. 2001). High levels of cytoplasmic GSH maintain cytochrome *c* in a reduced or inactive state. During oxidative stress cytochrome *c* is released from mitochondria and oxidized. The accumulation of oxidized form of cytochrome *c* in the cytoplasm can lead to apoptosis (Hancock et al. 2001).

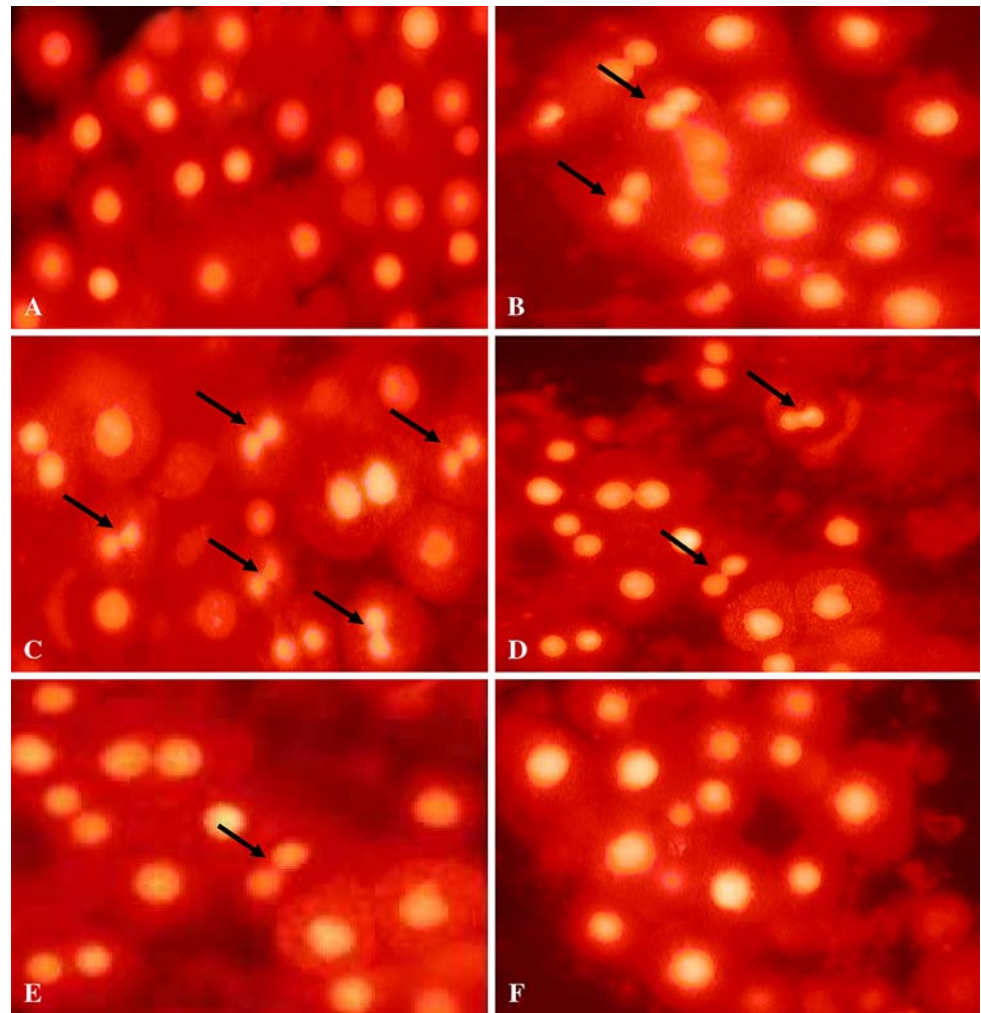
Improved hepatocyte viability, decreased mitochondrial susceptibility to swell, prevention of DNA damage, and reduced apoptosis in the presence of taurine could obviously be attributed to its ability to abrogate oxidative stress. Some studies suggest that taurine functions as a direct antioxidant to scavenge free radicals (Gordon et al. 1986; Chen 1993). Although this direct effect has been questioned by some investigators due to lack of structure–function

relationship (Wright et al. 1986; Halliwell and Gutteridge 1990), taurine is still considered to be an antioxidant by its capacity to halt the progression of oxidant-generating reactions. This includes upregulation of antioxidant system, formation of chloramines with hypochlorous acid and replenishment of GSH during oxidative stress. Taurine has been shown to be very effective in reducing lipid peroxidation induced by a variety of toxins such as kainic acid (Baran 2006), carbon tetrachloride (Miyazaki et al. 2005), tamoxifen (Tabassum et al. 2006), and thioacetamide (Balkan et al. 2001).

Besides these functions, taurine can preserve the structural and functional integrity of the cells by acting as a membrane stabilizer, osmoregulator, and a regulator of calcium homeostasis (Milei et al. 1992; Chen 1993). Taurine is cytoprotective to LLC-PK₁ cells exposed to dopamine and iron in vitro (Eppler and Dawson 2002). Taurine protects cultured cells under hypoxia and reoxygenation by reducing osmoregulatory dysfunction and improving intracellular calcium concentration (Michalk et al. 1996). Thus the protective effect of taurine on mitochondria might be related to the osmoregulatory effects and through Ca²⁺ homeostasis. Further studies to measure calcium fluxes and the effects of calcium on swelling and DNA damage are needed to address the action of taurine in the current study. Furthermore, taurine may enhance the anti-inflammatory response by suppressing the activation of nuclear factor- κ B (NF- κ B) probably by stabilizing the binding protein inhibitor of NF- κ B (I- κ B). Taurine along with niacin blocks lung fibrosis by inhibiting NF- κ B activation and preservation of I- κ B (Gurujeyalakshmi et al. 2000). Taurine and its chloramine can modulate the expression and production of pro-inflammatory substances (Schuller-Levis and Park 2004).

Inhibitors of CYP2E1 are suggested to be of therapeutic value in the treatment of alcoholic liver disease (Morimoto et al. 1995). Taurine by conjugation with cholic acid forms taurocholic acid which has been shown to be a potent inhibitor of CYP2E1 (Kerai et al. 1998). In addition, taurine could reduce iron action by chelation (Wu et al. 1999). Taurine has been shown to form complexes with free metal ion species such as Fe²⁺, Cu²⁺ with the sulfonic acid group (Trachtman et al. 1992) and works as a chelator at least, in vitro. The benefits may be possibly related to the observation that taurine could scavenge cytotoxic quinones generated as a result of iron-induced oxidation in vivo (Dawson et al. 2000). It is imperative to test whether taurine could perform such a function in vivo. Dietary taurine curtails myocardial iron level, oxidative stress, apoptosis, and fibrosis and replenishes reduced GSH level in iron-overloaded mice. Inhibition of L-type Ca²⁺ channels that are responsible for iron-uptake and regulation of redox cycling of iron has been proposed as

Fig. 4 Propidium iodide staining of isolated rat hepatocytes in EtOH, EtOH + Fe-treated liver cells in the absence and presence of taurine. Apoptotic cells with fragmented nuclei (*arrows*) are seen in EtOH, EtOH + Fe-treated cells (**b, c**). Hepatocytes isolated from ethanol, ethanol plus iron-treated rats coadministered with taurine treated-rats (**d, e**) show reduced apoptotic bodies while hepatocytes from control + taurine (**f**) show similar morphology as that with control (**a**). Magnification $\times 40$



the mechanism for reduction in iron content by taurine by Oudit et al. (2003).

Administration of taurine prevents apoptosis by increasing the level of GSH, a critical determinant of apoptosis and an important factor in regulating the viability of hepatocytes. Taurine stabilizes the GSH-metabolizing enzymes (Hayes et al. 2001), stimulates glucose-6-phosphate dehydrogenase, an enzyme that generates NADPH needed for the regeneration of GSH from GSSG (Tabassum et al. 2006), and modulates GSH levels. Taurine stimulates S-nitrosylation of GSH producing S-nitrosoglutathione, which is more potent than the GSH. S-nitrosylation of cysteine residues by nitrosoglutathione can inactivate caspase-3, thus preventing hepatic cell apoptosis (Chiueh and Rauhala 1999).

Studies in ischemic models have identified the underlying mechanisms of inhibition of apoptosis by taurine. One study observed that taurine suppresses the formation of Apaf-1/caspase-9 apoptosome and interaction of Apaf-1 with caspase-9 (Takatani et al. 2004). Recently, Taranukhin et al. (2008) identified that taurine reduces ischemia

induced caspase-8 and caspase-9 expression, the key inducers of apoptosis. Derivatives of taurine also exert downregulating effect on the apoptosis process (Marzioni et al. 2003). Further, taurine prevents hepatic DNA fragmentation and hepatocyte necrosis in acetaminophen-induced hepatotoxicity (Waters et al. 2001)

In our study, taurine, even when ethanol administration was continued, showed its protective effect suggesting that it could abolish the effects of alcohol. Recently, we showed that taurine enhances the metabolism and detoxification of ethanol, and prevents hepatic fibrosis in this model (Devi et al. 2008). In the same study we reported, histological changes induced in this model are reduced by taurine as evidenced by hematoxylin and eosin, and reticulin staining. Although several possible mechanisms seem to explain taurine's action, additional studies to elucidate the exact mechanisms of action are essential.

Allopathic treatment of alcoholic liver disease is primarily based on supplementation of nutraceuticals like protein hydrolysate, amino acids, vitamins, and trace elements. Taurine could be an useful adjunct for the treatment

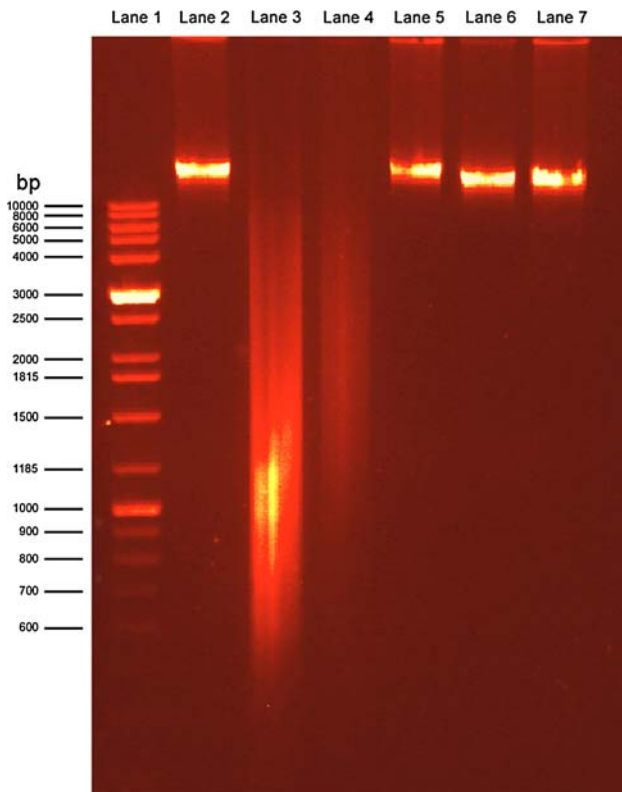


Fig. 5 DNA fragmentation pattern in liver of control and experimental animals. *Lane 1* molecular weight marker, *lane 2* CON, *lane 3* EtOH + Fe, *lane 4* EtOH, *lane 5* EtOH + Fe + taurine, *lane 6* EtOH + taurine, and *lane 7* CON + taurine

of alcoholic liver fibrosis considering the safety profile and the fact that humans have low capacity to synthesize taurine.

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