Alteration in Membrane Protein, Antioxidant Status and Hexokinase Activity in Erythrocytes of CCl_4- Induced Cirrhotic Rats

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Abstract- Several studies have shown that hepatocyte membrane composition changes in patients with cholestasis and cirrhosis. These alterations that are because of intracellular oxidative stress are supposed to be reflected in erythrocyte membrane. The aim of this study was to investigate the modification of erythrocyte membrane along with hexokinase and antioxidant enzymes during development of cirrhosis. Cirrhosis was induced by intraperitoneal injection of CCl_4 in male Wistar rats. The test groups were: baseline, cholestatic, early cirrhotic and advanced cirrhotic along with an equal number of sham-control animals. The erythrocyte membrane modifications (protein sulfhydryl, protein carbonyl, and lipid peroxidation), as well as NO metabolites, were assessed. Activities of GPX, CAT, SOD and HK were also measured. Protein sulfhydryl content of the erythrocyte membrane (after 2, 6 and 10 weeks of injection) had significant progressive decrease. In contrast, protein carbonyls were remarkably increased 2 weeks after injection but significantly decreased after 6 weeks and returned to normal levels after 10 weeks. No significant difference in erythrocyte HK activity or MDA content was observed. Test groups showed significantly lower erythrocyte GPx activity after six weeks and CAT and SOD activities along with NO metabolites content after two weeks (P<0.05). This study indicates that the progression of cirrhosis is accompanied by alterations in antioxidant enzyme and decreased NO metabolites. Protein carbonyl alteration occurs in the early stages of cirrhosis while protein sulfhydryl alterations have a progressive decrease in advanced cirrhosis.

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Keywords: Rats; Erythrocytes; Cholestasis; Liver cirrhosis; Oxidative stress; Protein carbonylation

Introduction

Cirrhosis is the third most common cause of death among people aged 45- 65 years (1). Chronic liver injury, irrespective of its cause, is generally associated with an accumulation of matrix proteins, a process referred to as fibrosis. In parallel with the continued stimulus for regeneration, further distortion of the hepatic architecture and vascular structures (portal veins, hepatic veins) occurs. This results in transformation to a nodular architecture so-called cirrhosis (2). Hepatic fibrosis, can be further complicated by hepatocellular carcinoma which is the fifth most common neoplasm, the major cause of death in patients with liver cirrhosis and the third most common cause of cancer-related death in the world (3,4). Oxidative stress is a disturbance in the oxidant-antioxidant balance leading to potential cellular damage (5). Cells have developed antioxidant systems which convert oxidants into non-toxic molecules, protecting the organism from deleterious effects of oxidative stress (6). A variety of enzymatic and nonenzymatic mechanisms have evolved to protect cells against reactive oxygen species (ROS). The enzymatic part include superoxide dismutase (SOD) that detoxifies superoxide ion, and catalase (CAT) and glutathione peroxidase (GPX) which detoxify cellular peroxides. Because of antioxidant capacity, most cells can tolerate a mild degree of oxidative stress, but severe conditions can damage cells (5). Oxidative stress contributes to the pathogenesis of acute and chronic

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liver diseases, hemochromatosis, alcoholic liver injury, toxin exposures, and even viral hepatitis (7). Lipid peroxidation, mediated by reactive oxygen species, leads to accumulation of cytotoxic products such as malondialdehyde (MDA) which impairs various cellular functions (7,8). Proteins are also major targets of ROS and their oxidation often leads to loss of their specific function. Oxidation of side chains to carbonyl residues is irreversible. Therefore, presence of carbonyl groups in proteins has been used as a marker of ROS-mediated protein oxidation (9). Oxidative stress also contributes to hepatic endothelial dysfunction by modulating nitric oxide (NO) bioavailability in the intrahepatic microcirculation (10). Increased resistance to portal blood flow is the primary factor in the pathophysiology of portal hypertension, the main complication of cirrhosis (11). Increase in hepatic vascular tone can be partly attributed to reduced NO bioavailability within liver (12). Because of the importance of oxidative stress in the pathogenesis of cirrhosis, measurement of systemic markers of oxidative stress has been suggested to reflect levels of oxidative stress present in the liver (7). The aim of this study was to assess protein sulfhydryls and carbonyls of erythrocyte membrane along with lipid peroxidation during development of cirrhosis and elucidate that if these probable alterations reflect stage of cirrhosis. In this regard authors also aimed to assay biomarkers of oxidative stress and antioxidants such as CAT, SOD, GPX, and GSH in erythrocytes, plasma total antioxidant capacity and NO metabolites (nitrite-nitrate) in carbon tetrachloride-induced cirrhotic rats during progression of liver damage.

Materials and Methods

Chemicals

Thiobarbituric acid (TBA) and (5,5′-dithiols-(2-nitrobenzoic acid)) (DTNB) were obtained from Sigma Chemical Co (St Louis, MO, USA). FeSO4.7H2O and Carbon tetrachloride (CCl4) were purchased from Riedel-de Haen, Germany. Tripyridyltriazine (TPTZ), trichloroacetic acid (TCA), 2, 4-dinitrophenylhydrazine (DNPH), vanadium (III) chloride, zinc sulfate, sulfanilamide (SA), N-(1-Naphthyl) ethylenediamine-dihydrochloride (NEDD), sodium nitrite and formaldehyde were obtained from Merck (Germany).

Male Wistar rats are weighting 200- 250 g were kept on standard rat chow with free access to tap water and in temperature and humidity controlled animal quarters with a 12-h light-dark cycle. All procedures were performed in accordance with the Animal Care Guidelines published by the National Institutes of Health in USA. Cirrhosis was induced in animals by injection of CCl4 (13,14). A total of rats were divided into four groups: 1- baseline or untreated controls (at the beginning of study), 2- cholestasis (after 2 weeks), 3- early cirrhotic (after 6 weeks), and 4- advanced cirrhotic (after 10 weeks). For groups 2, 3, and 4 equal numbers of sham-control animals were prepared. Test groups received CCl4 (100 µl/rat in 1 ml of mineral oil) three times a week for 2- 10 weeks through experimental period. Control groups received equivalent volumes of the solvent three times a week. The animals were sacrificed after 0, 2, 6 and 10 weeks of CCl4 injection, a period at which a complete development of cholestasis, cirrhosis and liver biochemical changes was clearly established. Blood and liver tissue samples were collected.

Blood collected from animals in heparin-containing tubes was centrifuged at 3000 rpm for 10 min at 4 °C. Aspiration and plasma removed plasma, and buffy coat was kept frozen at −80 oC for analysis. Activities of alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), and gamma-glutamyl transferase (GGT) were measured using a commercially available kit (Pars Azmun, Iran). Determination of Nitrate and Nitrite in plasma samples as an index of NO production was performed according to Griess method (15).

Plasma total antioxidant capacity was determined according to Benzie and Strain (16). This method is based on the ferric reducing ability of plasma (FRAP) which is estimated from the reduction of a ferric-tripyridyltriazine complex to the ferrous form at low pH. The absorbance of the resulting blue color was measured at 593 nm, and the total antioxidant capacity of plasma was determined using a standard curve.

Osmotic fragility (OF) of erythrocytes was determined by the method of Parpart *et al*. (17). Mean corpuscular fragility was calculated by recording saline concentration, which would have resulted in 50% hemolysis.

Erythrocyte membrane was isolated according to Dodge *et al*., with slight modifications (18). Briefly, erythrocytes were washed three times with cold (4 °C) phosphate-buffered saline (155 mM, pH 7.4). Erythrocyte ghosts were prepared following hypotonic lysis in cold 5 mM sodium phosphate buffer (pH 8.0) and centrifugation for 30 min at 4 °C at 25000 rpm. The supernatant (hemolysate) was decanted carefully, and pellet was washed three times. Total protein content of

the erythrocyte membrane was estimated by Biuret method with bovine serum albumin (BSA) as a standard (19).

Lipid peroxidation was estimated by measuring malondialdehyde (MDA) formation (20). Briefly, 0.1 ml of packed erythrocytes was suspended in 1.0 ml of reagent containing 15% w/v trichloroacetic acid, 0.375% w/v thiobarbituric acid and 0.25 N HCl. The mixture was heated in a water bath for 15 min at 85–90 °C. After cooling, the precipitate was removed by centrifugation. MDA concentration was calculated using its molar absorption coefficient of 1.56×105 M-1cm-1 and was expressed as nmol/mg protein.

Protein carbonyl content in erythrocyte membrane was determined using 2,4-dinitrophenylhydrazine (DNPH) assay with slight modifications (21). Erythrocyte membrane protein (0.5 mg) was precipitated with 10% trichloroacetic acid (4:1, v/v) and centrifuged at 4 °C for 5 min at 11000×g. Clear supernatant was discarded and the pellet was resuspended in 500 µl of 10 mM DNPH in 2 M HCl and allowed to stand at room temperature for 60 min, vortexing every 10-15 min to facilitate the reaction with proteins. Then, the protein was precipitated again with 50% trichloroacetic acid and centrifuged at 4 °C for 5 min at 11000×g. After washing the pellet with 500 µl ethanol:ethyl acetate (1:1, v/v), it was dissolved in 0.6 ml of 6 M guanidine hydrochloride at 37 °C and the insoluble materials were removed by centrifugation. Absorbance of the sample was measured against complementary blank at 370 nm. Carbonyl group content was calculated using the molar absorption coefficient of 2.2×104 M-1cm-1 and expressed as nmol/mg protein.

Erythrocyte membrane protein sulfhydryl content was determined using the Ellman reagent containing 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB) (22). Briefly, after denaturation of erythrocyte membrane protein with sodium dodecyl sulfate, it was reacted with Ellman reagent. The absorbance of the resulting thiophenylate anion was measured at 412 nm, and the sulfhydryl content was calculated using molar absorption coefficient of 1.36×104 M-1cm-1 and was expressed as µmol/mg protein.

Hemolysate was prepared from washed erythrocytes using ice-cold phosphate-buffered saline (155 mM, pH 7.4). Concentrations of hemoglobin in the hemolysate were measured by Drabkin's method (23). Hexokinase activity was measured using an assay kit from the Biomedical Research Service Center, SUNY Buffalo (Buffalo, NY), according to the manufacturer's instructions. CAT activity was assayed using CAT Assay Kit (BioVision, Mountain View, CA, USA). GPX and SOD activities were measured by commercially available kits (Ransel, Randox Lab, Crumlin, UK, respectively). Concentration of reduced GSH in each sample was estimated using DTNB, which develops a yellow color complex with GSH, with maximum absorption at 412 nm (24). Concentration of GSH was expressed as µmol/g Hb.

Liver tissue samples were taken immediately after sacrificing rats and fixed overnight in 3.7% formaldehyde solution, followed by fixing in 70% alcohol, 100% alcohol and xylene. After embedding in paraffin, samples were sectioned and stained with hematoxylin-eosin (H&E) reagent. The liver sections were studied under light microscope in double-blind examinations. Results are expressed as mean ± standard deviation (SD). Differences between groups were compared by analysis of variance (ANOVA). A *P*.value of ≤ 0.05 was considered statistically significant.

Results

Biochemical parameters

Biochemical parameters (liver enzyme activities) of the baseline control, sham-control and test (CCl_4) groups are shown in Table 1. Plasma activities of AST, ALT, ALP and GGT, were significantly higher in the test group than those in the baseline control and sham-control groups ($P<0.05$). Two weeks after CCl_4 treatment, total protein content of erythrocytes membrane significantly increased compared to those in the baseline control group and returned back near to normal values after 10 weeks.

Activity of antioxidant enzymes

Two weeks after CCl_4 treatment, activity of SOD significantly decreased in erythrocytes but returned to normal after six weeks. Cirrhotic rats and related sham-control groups showed significantly lower erythrocyte CAT activity in the 2nd week compared to that of baseline control group ($P<0.05$) and also in the 6th and 10th weeks. Six weeks after CCl_4 treatment, GSH content in test group significantly decreased compared to those of baseline control and sham-control groups ($P< 0.05$). Furthermore, cirrhotic rats showed a decrease in erythrocyte GPX activity in the 10th week compared to that of baseline control group ($P<0.05$). No significant difference in erythrocyte HK activity or plasma FRAP was observed between the baseline control and test groups.

Table 1. Biochemical parameters of control and test groups

Parameter	0th Week	2nd Week		6th Week		10th Week	
	Baseline Control	Control	Test	Control	Test	Control	Test
ALT (U/l)	36.29±6.02	33.80±9.62	325.94±84.4	134.26±17.96	236.56±122.13	33.64±3.95	254.70±81.60
ALP (U/l)	340.30±123.87	179.39±53.78	454.5±91.34^{bc10}	358.55±121.93	777.13±107.99^{abc10}	170.16±47.94	890.76±74.55^{ab}
AST (U/l)	51.55±6.29	49.28±12.74	372.21±64.12^{ab}	47.77±8.29	357.32±67.70^{ab}	127.22±39.36	294.0±131.79^{ab}
GGT (U/l)	2.75±0.88	3.2±1.80	6.54±2.07	3.51±1.13	7.87±3.17^{ab}	4.32±1.78	10.64±4.78^{ab}
Hb (gr/dl)	10.85±1.12	12.72±0.558	13.08±1.54^{a}	13.28±0.994	13.12±1.03^{a}	12.00±0.951	12.49±1.675
Pro (gr/dl)	1.06±0.24	1.44±0.20	1.52±0.19^{ac6,10}	1.45±0.22	1.18±0.27^{c2}	0.94±0.17	1.13±0.23^{c2}

Control group (Control), CCl_4-treated rats (Test), each value represents mean ± SD (n=7) and experiments performed in duplicate. AST: Aspartate aminotransferase; ALT: Alanine amino transaminase; GGT: Gamma glutamyl transferase; ALP: Alkaline phosphatase; Hb: Total hemoglobin; Pro: Total protein. [a]Significantly different from baseline control, P value is less than 0.05. [b]Significantly different from sham-control, P-value ≤ 0.05. [c]Significantly different from carbon tetrachloride (CCl_4)-treated rats (Test) group, *P-value* ≤ 0.05

Table 2. Erythrocyte antioxidant enzyme activities, reduced glutathione (GSH), glutathione total, hexokinase (HK), plasma Nitric Oxide (NO) metabolites and ferric reducing ability of plasma (FRAP) of control and test (CCl_4) groups

Parameter	0th Week	2nd Week		6th Week		10th Week	
	Baseline Control	Control	Test	Control	Test	Control	Test
CAT (nmol/min/ml)	10.71±4.12	3.91±2.03	4.07±1.18^{a}	6.07±1.99	4.71±3.1^{a}	4.84±2.88	4.34±1.38^{a}
SOD (U/gr Hb)	95.92±28.90	85.80±10.20	52.56±24.31^{abc10}	73.40±6.61	78.84±9.97	75.19±7.90	88.00±16.41^{c6}
GPX (U/gr Hb)	385.24±71.93	298.76±71.78	398.49±50.66^{b}	332.20±43.28	385.16±25.30	207.72±20.81	309.22±46.43^{b}
GSH (µmol/gr Hb)	449.35±64.37	439.14±107.56	451.96±118.17^{c6}	426.82±74.37	264.79±64.10^{abc2}	292.96±44.32	335.12±29.86
FRAP (µmol/l)	328.31±83.93	418.73±136.63	498.63±165.60	360.67±120.29	380.97±128.39	520.47±110.42	390.54±77.65
NO metabolites (µmol/l)	17.62±3.02	23.51±4.78	18.69±1.92^{c6}	19.93±4.08	11.36±5.19^{abc2}	17.18±3.17	15.20±2.84
HK (miliU)	0.216±0.037	0.187±0.059	0.178±0.027	0.231±0.027	0.238±0.040	0.201±0.058	0.174±0.061

GPX: Glutathione peroxidase; CAT: Catalase; SOD: Superoxide dismutase; GSH: Reduced glutathione; HK: Hexokinase; NO: Nitric Oxide metabolites; FRAP: Ferric reducing ability of plasma. Significantly different from baseline control, $P< 0.05$. [b]Significantly different from sham-control, $P< 0.05$. [c]Significantly different from carbon tetrachloride (CCl_4)-treated rats (Test group), $P< 0.05$. Each value represents mean±SD (n=7), and the experiments were performed in triplicates

As shown in Table 2, plasma NO metabolites concentration was significantly lower in the test group than that in the baseline control group after six weeks of treatment ($P<0.05$).

Membrane protein modifications

Protein sulfhydryl concentration in erythrocyte membranes was significantly lower in CCl4 treated rats 2, 6 and 10 weeks after injection compared to baseline control rats (Figure 1). In contrast, protein carbonyls were remarkably increased 2 weeks after injection but significantly decreased after 6 weeks and returned to normal levels after 10 weeks (Figure 2).

As shown in Figure 3, no significant difference in erythrocyte membrane MDA content was observed between baseline control and Test groups. In order to measure the effect of CCl_4 treatment on erythrocyte membrane integrity, the mean NaCl concentrations corresponding to 50% hemolysis of the cell was expressed as mean erythrocyte fragility (MEF) in various groups. The concentrations of NaCl corresponding to 50% hemolysis of erythrocytes for each treatment were calculated by extrapolating the results shown in Figure 4.

Figure 5 shows the alteration in osmotic fragility of erythrocytes during progression of cirrhosis. A Significant association between MEF and severity of cirrhosis was observed.

Histological study

Figure 6 shows the hematoxylin- Eosin stained sections of the liver. Panel A represents a control tissue where the architecture of the hepatic parenchyma seems normal. Panel B shows a test sample after two weeks in which some slight areas of necrosis is observed, and granules of fat can be seen. Panel C corresponds to the liver from CCl_4 treated animals after six weeks; in this case infiltration of inflammatory cells has happened, and the cells are surrounded by blood. Panel D corresponds to the liver from CCl_4 treated animals after 10 weeks that necrosis and inflammation is evident, and many inflammatory cells are present in the area. These histopathological events are in line with the biochemical changes, and prove the cirrhosis progression in CCl_4 treated animals after six weeks.

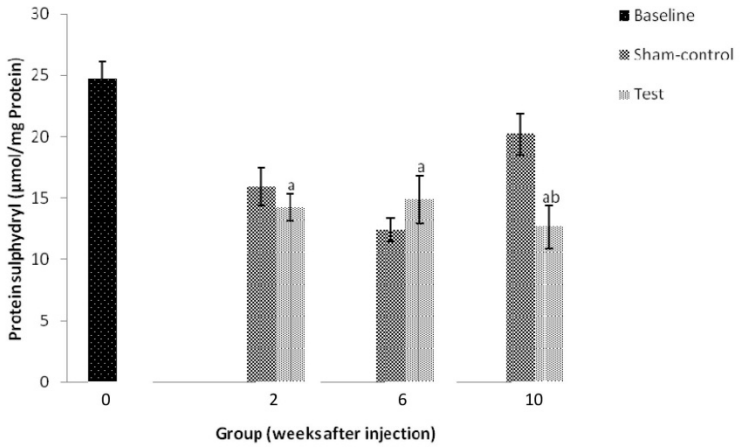

Figure 1. Erythrocyte membrane protein sulfhydryl content in baseline control, sham-control and CCl_4-treated rats (Test groups). Each value represents mean±SD (n=7). All assays were carried out in triplicate. 'a' significantly different from baseline control, $P<0.05$. 'b' significantly different from sham-control group, $P<0.05$

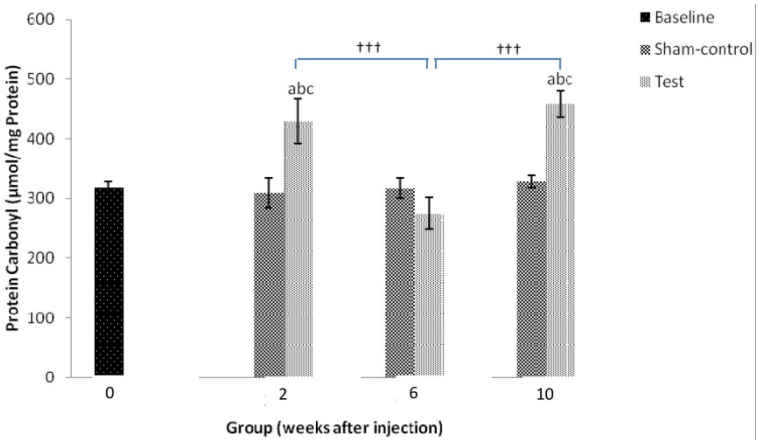

Figure 2. Erythrocyte membrane protein carbonyl content in baseline control, sham-control and CCl_4-treated rats (Test groups). Each value represents mean±SD (n=7). All assays were carried out in triplicate. 'a' significantly different from baseline control, $P<0.05$. 'b' significantly different from sham-control, $P<0.05$. 'c' significantly different from carbon tetrachloride (CCl_4)-treated rats (Test groups), $P< 0.05$

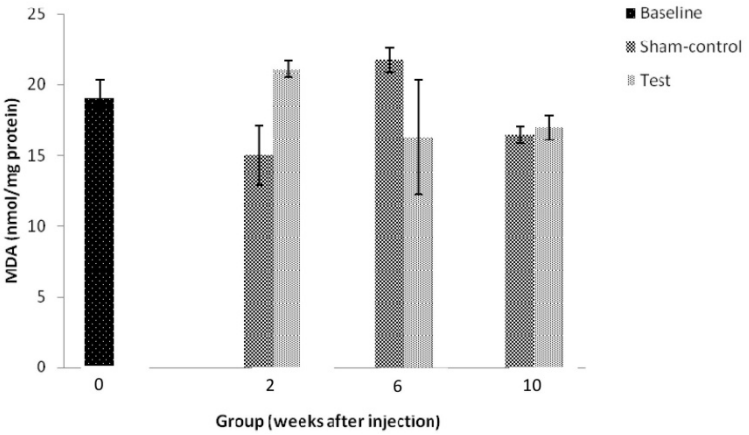

Figure 3. Erythrocyte membrane lipid peroxidation determined as malondialdehyde (MDA) content in baseline control, sham-control and CCl_4-treated rats. Each value represents mean±SD (n =7). All assays were carried out in triplicate

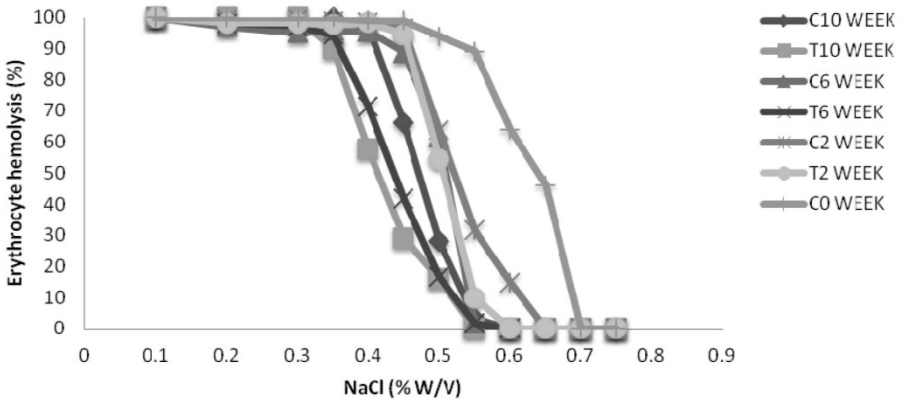

Figure 4. Alteration in osmotic fragility of erythrocyte membrane in CCl_4-treated rats and baseline control

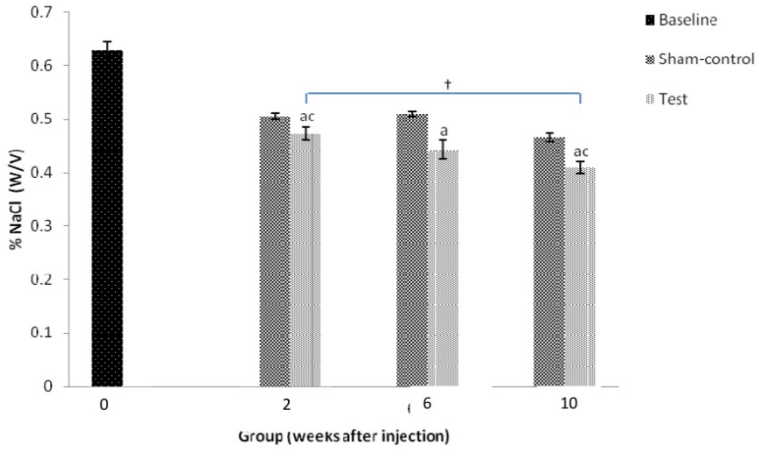

Figure 5. Mean erythrocyte fragility (MEF) determined in baseline control, sham-control and carbon tetrachloride (CCl_4)-treated rats (Test). Each value represents the mean value of experiments performed in duplicate±SD (n=7). 'a' significantly different from baseline control, $P<0.05$. 'b' significantly different from carbon tetrachloride (CCl_4)-treated rats (Test) group, $P< 0.05$

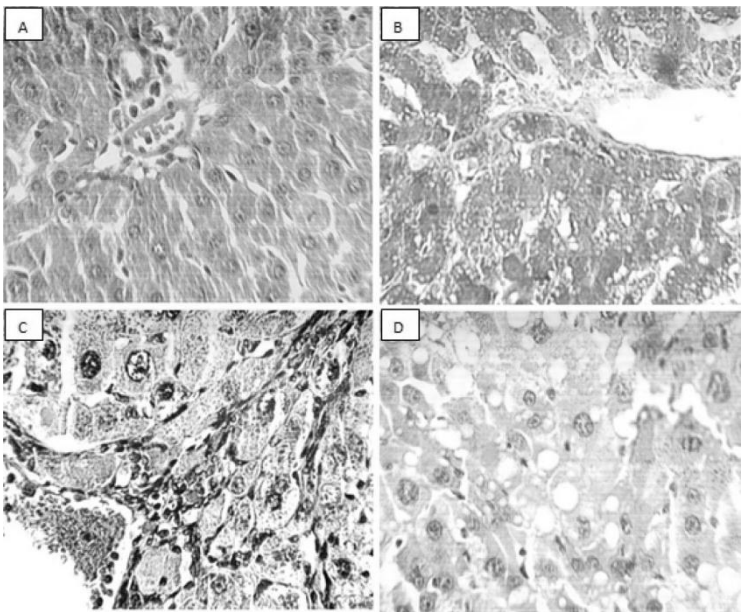

Figure 6. Hematoxylin-Eosin staining of liver tissue from: (a) Control rats, (b) CCl_4-treated rats after 2 weeks, (c) CCl_4-treated rats after 6 weeks, (d) CCl_4-treated rats after 10 weeks

Discussion

The results of current study show that CCl_4-induced cirrhosis is accompanied by oxidative stress in liver and erythrocytes. CCl_4-induced fibrosis and cirrhosis is one of the oldest and probably the most widely used toxin-based experimental models for the induction of fibrosis. This model has the advantages that it is clearly characterized and in many respects mirrors the pattern of disease seen in human fibrosis and cirrhosis associated with toxic damage (25). CCl_4-induced liver damage was evident by a significant increase in the enzymatic activities of AST, ALT, ALP, and GGT in serum which has been attributed to severe damage to the liver (26). The activities of the antioxidant enzymes (except for GPX at 2nd and 6th week), as well as the level of GSH, were decreased after CCl_4 treatment. It can be inferred that CCl_4-induced liver injury causes oxidative stress and diminution of antioxidant system capacity in erythrocytes. Antioxidant enzymes work in concert to detoxify superoxide anion and H2O2 in cells. Results of this study indicate that erythrocyte GPX activity was decreased in cirrhotic rats in the 10th week compared to that of the baseline control group ($P< 0.05$). Erythrocyte CAT activity in test groups was decreased 0.38-, 0.43- and 0.40-folds compared to that of baseline and sham-control group after 2, 6 and 10 weeks of treatment, respectively. SOD activity in erythrocytes of test groups was decreased 0.54-, 0.82- and 0.91-folds after 2, 6 and 10 weeks of injection compared to baseline control group. GSH content also was decreased 0.58- and 0.74-folds after 6 and 10 weeks of treatment. The results showed a significant decrease in erythrocyte activity of SOD after 2 weeks, GPX after 10 weeks and CAT from 2nd week and on, as well as GSH content after 6 and 10 weeks. Present results support in part the previous studies in this regard (27). The decreased activity of SOD in erythrocytes in CCl_4-treated rats may be because of the accumulation of ROS or inactivation of the antioxidant enzymes. This would cause an increased accumulation of superoxide radicals, which would further stimulate lipid peroxidation after two weeks although its increase is not significant. Decrease in GPX activity after 10 weeks of CCl_4 toxicity might be due to decreased availability of GSH resulted from enhanced production of ROS. These data suggests that decreased compensatory ability of antioxidant defense system may be the result of increased oxidative stress due to pathological changes in cirrhosis. It is reported that when capacity of cells and tissues to maintain GSH homeostasis is diminished, cell injury often follows, and GSH depletion and loss of protein thiols precede cell death (28). Erythrocytes are particularly vulnerable to oxidative damage because of high contents of polyunsaturated fatty acids in their membranes leading to the formation of the lipid peroxidation product; malondialdehyde (MDA). Reactive oxygen species work against proteins and modify amino acid residues (lysine, arginine, proline, and histidine) generating carbonyl moieties, which is identified as an early marker for protein oxidation and is used as a measure of protein damage (21). Oxidative damage of membranes results in increased membrane fluidity, compromised integrity, and inactivation of membrane-bound receptors and enzymes (29). The results showed that CCl_4 induction of oxidative stress in erythrocyte, caused a slight increase in the MDA level after 2 weeks and significant increase in protein carbonyl group content after 2 and 10 weeks, as well as significant progressive decrease in protein sulfhydryl groups content after 2, 6 and 10 weeks. It seems that there is a relation between decrease in protein sulfhydryl group and a significant increase in protein carbonyl group content of the membrane. Some oxidative modifications are quite specific, both in the oxidized residue and the product generated; others can alter multiple residues and may rise several products. It had been shown that carbonyl group introduction into side chains of Pro, Arg, Lys, and Thr residues can be regarded as an example of a global modification of proteins; this can arise from subsequent reaction with the primary oxidation products such as 4-hydroxy-2-nonenal (30). The study on osmotic fragility showed that haemolysis began in less hypotonic solution in the RBCs of CCl_4-treated rats which shows that these erythrocytes are more fragile than that of baseline control rats. This effect may partly be because of membrane alterations observed in terms of cholesterol/phospholipid ratio and ATPase activities (31). These modifications of membrane components can be attributed globally to altered oxidative situation in erythrocytes of cirrhotic rats. In this study, authors have observed that OF alterations were in accordance with oxidative stress in cirrhotic rats and thus can be considered as a potential biomarker of oxidative membrane damage in pathologic conditions.

NO derived from vascular endothelial cells is crucial for normal vasoregulation and is likely to have a similar role in hepatic microvascular homeostasis (32). The results about NO metabolites in this study are similar to those reported recently in the bile duct ligation model of

liver injury (33). The present investigation shows that NO metabolites are diminished in CCl_4-induced cirrhosis that may be due to decreased hepatic eNOS activity.

Magnani *et al*., showed that hexokinase is the major regulatory enzyme for the pentose phosphate pathway during oxidative stress. In addition, NADPH produced in this process guarantees the gluthatione redox system homeostasis and provides substrates for antioxidative defense system against oxidative injury. So, increased hexokinase activity in erythrocyte (after sixth week) can be considered as a compensative response against oxidative stress which results in increase of pentose phosphate efficiency and a stabilizing factor of redox situation inside cell (34).

Present study indicates that protein carbonyls modification precedes the protein sulfhydryls and may be considered as a better marker for the early stages of cirrhosis, whereas protein sulfhydryls modification has a progressive descending trend in chronic phases. This may be accounted for an efficient marker for the late stages of the disease. It can be inferred that CCl_4-induced liver injury causes oxidative stress and over consumption of antioxidants in erythrocytes. The results show that the antioxidant capacity of erythrocytes is depleted in the early stages of cirrhosis.

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