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ORIGINAL ARTICLE

Erdosteine treatment attenuates oxidative stress and fibrosis in experimental biliary obstruction

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Abstract Oxidative stress, in particular lipid peroxidation, induces collagen synthesis and causes fibrosis. The aim of this study was to assess the antioxidant and antifibrotic effects of erdosteine on liver fibrosis induced by biliary obstruction in rats. Liver fibrosis was induced in Wistar albino rats by bile duct ligation (BDL). Erdosteine (10 mg/kg, orally) or saline was administered for 28 days. Serum aspartate aminotransferase (AST), alanine aminotransferase (ALT) and lactate dehydrogenase (LDH) levels were determined to assess liver functions and tissue damage, respectively. Pro-inflammatory cytokines, TNF-α, IL-1β and IL-6 and antioxidant capacity (AOC) were assayed in plasma samples. Liver tissues were taken for determination of malondialdehyde (MDA) and glutathione (GSH) levels, myeloperoxidase (MPO) activity and collagen content. Production of reactive oxidants was monitored by chemiluminescence assay. Serum AST, ALT, LDH, and plasma cytokines were elevated in the BDL group as compared to controls and were significantly decreased by erdosteine treatment. Hepatic GSH level and plasma AOC, depressed by BDL, were elevated back to control level with erdosteine treatment. Furthermore, hepatic luminol and lucigenin chemiluminescence (CL), MDA level, MPO activity and collagen content in BDL group increased dramatically compared to control and reduced by erdosteine treatment. Since erdosteine administration alleviated the BDL-induced oxidative injury of the liver and improved the hepatic functions, it seems likely that erdosteine with its antioxidant and antifibrotic properties, may be of potential therapeutic value in protecting the liver fibrosis and oxidative injury due to biliary obstruction.

Keywords Erdosteine · Bile duct ligation · Hepatic fibrosis · Lipid peroxidation

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Introduction

Chronic cholestatic liver diseases, including primary biliary cirrhosis, extrahepatic biliary arresia, idiopathic adulthood ductopenia, primary sclerosing cholangitis, idiopathic neonatal hepatitis, and arteriohepatic dysplasia, are leading indications for liver transplantation [1, 2]. On the otherhand various drugs, total parenteral nutrition, chronic liver transplant rejection, and graft-versus-host disease can also produce chronic cholestasis [3].

Cholestasis produced by a ligation of the common bile duct in rats induces a number of physiological changes, including obstructive jaundice, secondary liver cirrhosis, and fibrosis [4]. How cholestasis induces liver injury and fibrosis remains unclear. Hepatic fibrosis, the main complication of chronic liver disease,

is usually initiated by hepatocyte damage, leading to recruitment of inflammatory cells and platelets, activation of Kuppfer cells and subsequent release of cytokines and growth factors [5]. These factors probably link the inflammatory processes and oxygen free radicals, which are known to cause tissue-fibrosis [6, 7]. It is known that increased concentration of bile acids induce lipid peroxides, probably related to the stimulation of phagocytic activity in the polymorphonuclear leukocytes and inflammatory cells, which are present after biliary tract obstruction and enhance the tissue injury [8, 9]. Thus free radical ablation for the treatment of cholestatic liver injury might be useful in the prevention of fibrosis and oxidative damage following biliary obstruction.

Erdosteine is a thiol derivative that has recently been introduced into clinical practice as a well-tolerated mucoactive drug, used for its mucolytic and antioxidant effects [10]. Its molecule contains two sulphur atoms; one is present in an aliphatic side-chain and the other is enclosed in the heterocyclic ring [11]. These chemically blocked sulfydryl groups are liberated following hepatic metabolism and thereby the molecule subsequently exerts its free radical scavenging and antioxidant properties [12]. Based on its free radical scavenging activity, its protective effects against oxidant-induced tissue damage have been demonstrated in various inflammation models [13–16]. However, most of these studies have focused on the beneficial effects of erdosteine on chemotherapeutic drug-induced toxicity of the renal, pulmonary and cardiac tissues, but the protective effect of erdostein against cholestasis was not evaluated yet. Thus we speculate that erdosteine would provide protection on the liver tissues against the biliary obstruction induced oxidative damage and fibrosis in rats.

Materials and methods

Animals

Male Wistar albino rats (200–250 g) were housed in a room at a mean constant temperature of 22 ± 2°C with a 12 h light–dark cycle, and free access to standard pellet chow and water. The study was approved by the Marmara University School of Medicine Animal Care and Use Committee.

Induction of liver fibrosis

Rats were anaesthetized (100 mg/kg ketamine and 0.75 mg/kg chlorpromazine, i.p.) and the common bile duct was exposed and ligated by double ligatures with suture silk. The first ligature was made below the junction of the hepatic ducts and the second ligature was made above the entrance of the pancreatic ducts. Finally, the common bile duct was resected between the double ligatures [17]. In sham-operated rats, abdominal incision was made without a ligation.

Experimental groups

Saline (1 ml/rat) or erdosteine (10 mg/kg, Sandoz Ilac, Istanbul, Turkey) were given orally to sham operated (control and ERDO groups) and bile duct ligated (BDL and BDL + ERDO group) rats for 28 days. Each group consists of ten animals.

After 28 days of treatment, the rats were decapitated and trunk blood was collected. Serum samples were used for the measurement of bilirubin, aspartate transferase (AST), alanine transferase (ALT), and lactate dehydrogenase (LDH) levels, as a marker of hepatic function and tissue injury, respectively. Pro-inflammatory cytokines, tumor necrosis factor (TNF)-α, interleukin (IL)-1β, and IL-6, and antioxidant capacity were determined in plasma samples. Liver samples were taken and stored at –70°C for the measurement of malondialdehyde (MDA) and glutathione (GSH) levels, myeloperoxidase (MPO) activity and collagen contents. Formation of reactive oxygen species in liver samples was monitored by using chemiluminescence method. Tissues were also examined histologically.

Biochemical analysis

Serum AST, ALT [18] and LDH levels [19] were determined spectrophotometrically using an automated analyzer. Serum total bilirubin level was assayed on Bayer Opera Autoanalyzer according to a method described previously [20]. Plasma TNF-α IL-1β and IL-6 were quantified according to the manufacturer's instructions and guidelines using enzyme-linked immunosorbent assay (ELISA) kits specific for the previously mentioned rat cytokines (Biosource International, Nivelles, Belgium). These particular assay kits were selected because of their high degree of sensitivity, specificity, inter- and intra-assay precision, and small amount of plasma sample required to conduct the assay. The total antioxidant capacity in plasma were measured by using colorimetric test system (ImAnOx, cataloge no. KC5200, Immunodiagnostic AG, D-64625 Bensheim), according to the instructions provided by the manufacturer.

Chemiluminescence (CL) assay

To assess the role of reactive oxygen species in BDL-induced hepatic damage, luminol and lucigenin chemiluminescences were measured as indicators of radical formation. Lucigenin (bis-*N*-methylacridiniumnitrate) and luminol (5-amino-2,3-dihydro-1,4-phthalazinedione) were obtained from Sigma (St Louis, MO, USA). Measurements were made at room temperature using Junior LB 9509 luminometer (EG&G Berthold, Germany). Specimens were put into vials containing PBS-HEPES buffer (0.5 M PBS containing 20 mM HEPES, pH 7.2). ROS were quantitated after the addition of the enhancers, lucigenin or luminal, for a final concentration of 0.2 mM. Luminol detects a group of reactive species, i.e. $^{\bullet}OH$, H_2O_2, HOCl radicals, while lucigenin is selective for O_2^- (25, 26). Counts were obtained at 1 min intervals and the results were given as the area under curve (AUC) for a counting period of 5 min. Counts was corrected for wet tissue weight (rlu/mg tissue) [21, 22].

Malondialdehyde and glutathione assays

Tissue samples were homogenized with ice-cold trichloracetic acid (1 g tissue plus 10 ml 10% TCA) in an Ultra Turrax tissue homogenizer. The MDA levels were assayed for products of lipid peroxidation by monitoring thiobarbituric acid reactive substance formation as described previously [23]. Lipid peroxidation is expressed in terms of MDA equivalents using an extinction coefficient of 1.56×10^5/M/cm and the results are expressed as nmol MDA/g tissue. Glutathione measurements were performed using a modification of the Ellman procedure [24]. Briefly, after centrifugation at 2,000*g* for 10 min, 0.5 ml of supernatant was added to 2 ml of 0.3 mol/l$Na_2HPO_4{\cdot}2H_2O$ solution. A 0.2 ml solution of dithiobisnitrobenzoate (0.4 mg ml 1% sodium citrate) was added and the absorbance at 412 nm was measured immediately after mixing. Glutathione levels were calculated using an extinction coefficient of 1.36×10^5/M/cm. The results are expressed in μmol GSH/g tissue.

Measurement of liver myeloperoxidase (MPO) activity

Tissue samples (0.2–0.3 g) were homogenized in 10 vol of ice-cold potassium phosphate buffer (50 mmol/l K_2HPO_4, pH 6.0) containing hexadecyltrimethylammonium bromide (HETAB, 0.5%, w/v). The homogenate was centrifuged at 30,000*g* for 10 min at 4°C, and the supernatant was discarded. The pellet was then rehomogenized with an equivalent volume of 50 mmol/l K_2HPO_4 containing 0.5% (w/v) hexadecyltrimethylammonium bromide and 10 mmol/l ethylenediaminetetraacetic acid (EDTA, Sigma). MPO activity was assessed by measuring the H_2O_2-dependent oxidation of *O*-dianizidine·2 HCl. One unit of enzyme activity was defined as the amount of the MPO present per gram of tissue weight that caused a change in absorbance of 1.0/min at 460 nm and 37°C [25].

Measurement of liver collagen content

Tissue collagen was measured as a free radical-induced fibrosis marker. Tissue samples were cut with a razor blade, immediately fixed in 10% formalin then samples were embedded in paraffin, and sections, approximately 15 μm thick were obtained. The evaluation of collagen content was based on the method published by Lopez de Leon and Rojkind that is based on selective binding of the dyes sirius red and fast green to collagen and noncollagenous components, respectively [26]. Both dyes were eluted readily and simultaneously by using 0.1 N NaOH-methanol (1:1, v/v). Finally, the absorbances at 540 and 605 nm were used to determine the amount of collagen and protein, respectively.

Light microscopic preparation

For light microscopic investigations samples from the liver were fixed with 10% formaldehyde, dehydrated in graded alcohol series, cleared with toluen and embeded in paraffin. Tissue sections (5 μm) were stained with hematoxylin and eosin (H&E) for general morphology and the determination of fibrosis with Masson's trichrome, and examined under an Olympus BX51 photomicroscope (Tokyo, Japan).

Statistical analysis

Statistical analysis was carried out using GraphPad Prism 3.0 (GraphPad Software, San Diego CA, USA). All data were expressed as mean ± SEM. Groups of data were compared with an analysis of variance (ANOVA) followed by Tukey's multiple comparison tests. Values of $P < 0.05$ were regarded as significant.

Results

The fifth day after surgery, the cholestasis was evidenced by a significant increase in the levels of total bilirubin, which was still high at the 28th day after surgery (Table 1). As shown in Table 1, serum AST,

Table 1 Serum total biluribin levels,

Groups	C	ERDO	BDL	BDL+ ERDO
Total Bilirubin (mg/dl)	0.38 ± 0.1	0.42 ± 0.2	8.5 ± 1.7 ***	6.2 ± 0.5 ***, $^{+++}$
AST (mg/dl)	175 ± 5.4	181 ± 7.1	346 ± 20 ***	260 ± 16 *, $^{++}$
ALT (mg/dl)	67 ± 5.1	70 ± 5.3	127 ± 9.4 ***	72 ± 4.9 $^{+++}$
LDH (U/l)	1904 ± 25	1730 ± 32	2766 ± 112 ***	2250 ± 60 **, $^{+++}$
AOC (pg/ml)	577 ± 28.7	605 ± 23.2	309 ± 27.3 ***	518 ± 41.5 $^{+++}$

Each group consists of ten animals

AST aspartate aminotransferase, *ALT* alanine aminotransferase and *LDH* lactate dehydrogenase activity, and *AOC* plasma antioxidant capacity in control (*C*), *ERDO* erdosteine, *BDL* bile duct ligation and *BDL* + *ERDO* groups

$^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ compared with control group. $^{++}P < 0.01$, $^{+++}P < 0.001$ compared with *BDL* group

ALT, and LDH activity were significantly higher in BDL rats compared to controls ($P < 0.001$), while erdosteine administration in BDL group reduced these values ($P < 0.01$–0.001). On the other hand, the total antioxidant capacity in plasma was decreased significantly ($P < 0.001$) due to BDL, whereas erdosteine treatment in the rats with BDL increased this parameter significantly ($P < 0.001$).

In the saline-treated BDL rats, the levels of TNF-α, IL-1β, IL-6 were significantly increased when compared to control group ($P < 0.001$), while this BDL-induced rise in plasma cytokine levels was reduced significantly by erdosteine treatment ($P < 0.001$, Fig. 1).

Luminol and lucigenin CL values of the liver samples of the BDL group (15.3 ± 0.9 and 15.6 ± 0.8 rlu/mg, respectively) were found to be significantly higher than those of the control group (8.9 ± 0.9 and 11.2 ± 0.7 rlu/mg; $P < 0.001$ and $P < 0.01$, respectively) and erdosteine treatment reversed these values (9.4 ± 0.6 and 12.2 ± 0.9 rlu/mg, $P < 0.001$ and $P < 0.05$, respectively) (Fig. 2a, b).

The liver MDA was found to be significantly higher in the BDL group (84.8 ± 3.3 nmol/g, $P < 0.001$) compared to that of the control group (51.3 ± 1.8 nmol/g). Treatment with erdosteine reversed the MDA level (58.5 ± 1.9 nmol/g, $P < 0.001$) back to control level (Fig. 3a). The hepatic GSH level showed a marked reduction in the BDL group (1.3 ± 0.1 μmol/g, $P < 0.001$) compared to that of the control group (2.01 ± 0.1 μmol/g). Similarly, treatment with erdosteine reversed this effect (1.71 ± 0.1 μmol/g, $P < 0.05$) (Fig. 3b).

Liver MPO activity, which was observed to be higher in the BDL group (19.7 ± 1.4 U/g, $P < 0.001$) compared to control group (8.1 ± 1.1 U/g), was also attenuated by erdosteine treatment (11.1 ± 0.9 U/g, $P < 0.001$) (Fig. 4a). Hepatic collagen content of the saline-treated BDL rats (25.9 ± 2.6 μg/mg protein, $P < 0.001$) was found to be significantly higher than that of the control group (13.5 ± 0.5 μg/mg protein). Erdosteine treatment significantly reduced the increase in hepatic collagen content (15.5 ± 1.4 μg/mg protein, $P < 0.001$) to the levels that were close to control values (Fig. 4b).

Light microscopic evaluation of the control (Fig. 5a, b) group revealed a regular morphology of liver parenchyma with intact hepatocytes and sinusoids. However, in the BDL group, hepatic cords were disorganised and hepatocytes showed degeneration and increased eosinophilia. Fibrosis was evident in the form of delicate bands around the acini. Other findings in this group were bile duct proliferation and mononuclear inflammatory infiltration in the portal tract (Fig. 5c, d). On the other hand erdosteine treatment to the BDL rats caused decrease in the number of degenerated and eosinophilic hepatocytes with mild disorganisation of hepatic cords. Moreover, there were mild fibrosis around the acini and inflammatory cell infiltration within the portal tract (Fig. 5e, f).

Discussion

As confirmed by the biochemical and histological data, the results of the present study demonstrate that treatment with erdosteine markedly improves the BDL-induced hepatic damage in the rats. Moreover, all the parameters indicating the presence of oxidative injury were markedly reversed by erdosteine treatment, suggesting that erdosteine has a potent antioxidant and anti-inflammatory effect on the fibrotic tissue.

The liver is an important organ in the metabolism of cytokines with the capacity both to produce and to remove them [27]. Several reports point out an imbalance among pro- and anti-inflammatory and pro- and anti-fibrotic cytokines throughout human hepatic diseases. Serum levels of IL-1 β, IL-6, TNF-α, and interferon gamma (IFN-gamma), are elevated in

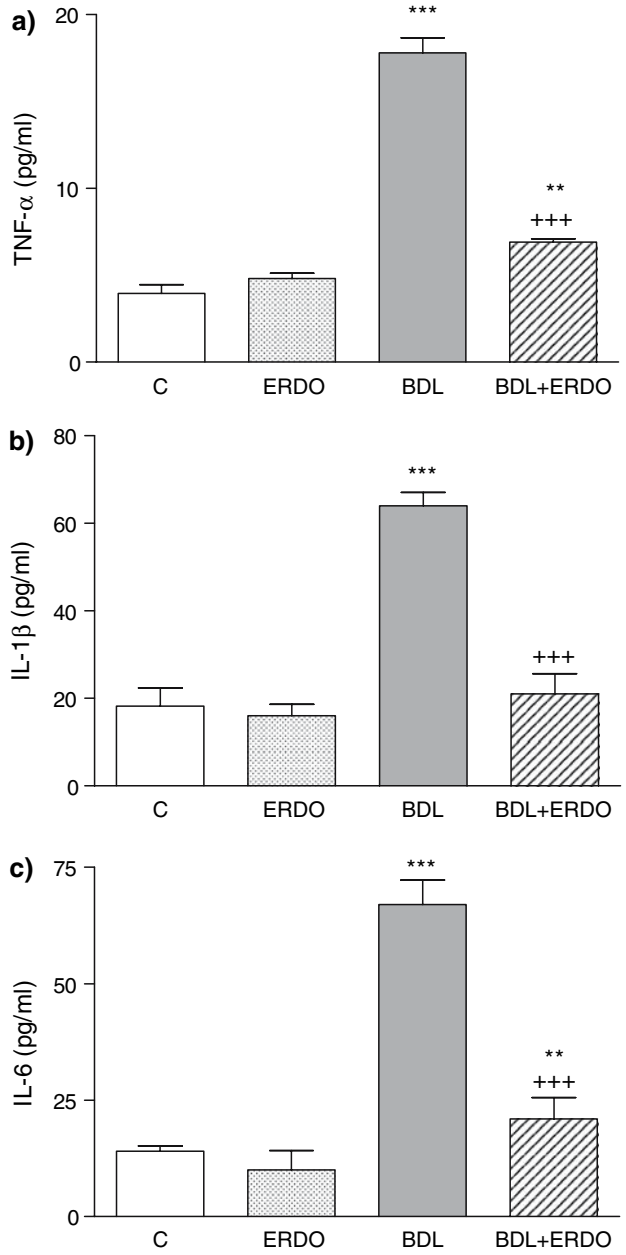

Fig. 1 Plasma **a** Tumor necrosis factor- alpha (TNF-α), **b** Interleukine 1-beta (IL-1β) and **c** IL-6 levels in the control *C*, *ERDO* erdosteine, *BDL* bile duct ligation and *BDL + ERDO* groups. Each group consists of ten animals. $^{**}P < 0.01$, $^{***}P < 0.001$ compared with control group. $^{+++}P < 0.001$ compared with *BDL* group

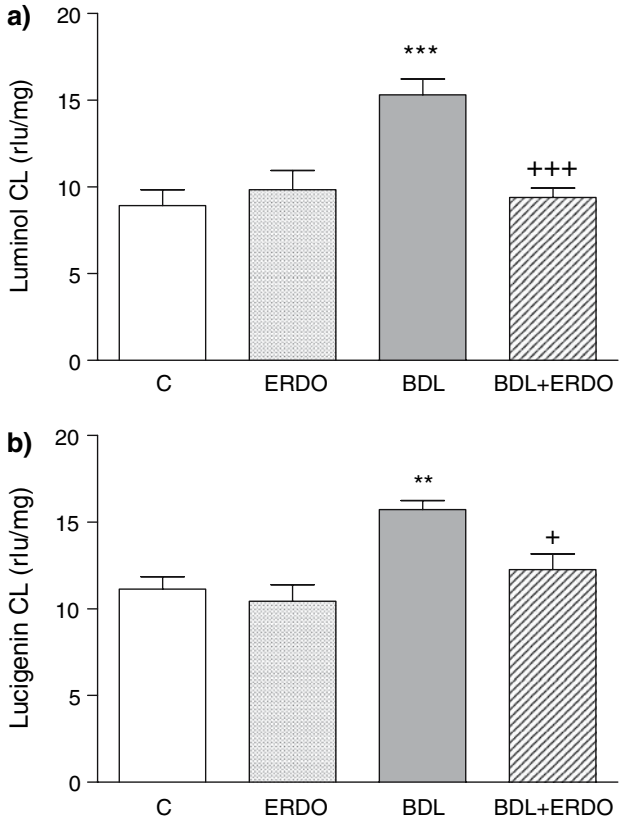

Fig. 2 **a** Luminol and **b** Lucigenin chemiluminescence values of the liver tissues in the control *C*, *ERDO* erdosteine, *BDL* bile duct ligation and *BDL + ERDO* groups. Each group consists of ten animals. $^{**}P < 0.01$, $^{***}P < 0.001$ compared with control group. $^{+}P < 0.05$, $^{+++}P < 0.001$ compared with *BDL* group

patients with chronic liver disease [28]. In the rat studies, chronic BDL significantly increased most of plasma and hepatic cytokines [29, 30]. Similarly in our study, TNF-α, IL-1 β, and IL-6 were significantly elevated due to BDL, while erdosteine treatment markedly reduced these cytokine levels. Cytokines, although play important roles in the normal physiology of cells and organs, are most distinguished for their activities associated with immune response, inflammation, tissue injury or repair and organ dysfunction [27, 31]. In the inflammatory human diseases, the extravasal recruitment of neutrophils, a major source of reactive oxygen radicals, plays a crucial role in the development of tissue damage, which, when persistent, can lead to the irreversible organ dysfunction [32]. Targeting oxidative stress with antioxidants or boosting the endogenous levels of antioxidants is likely to be beneficial in the treatment of the chronic inflammatory diseases. Thus, it is possible that compounds implicated in the pathogenesis of either inflammatory disease, may also be effective in the treatment of other chronic inflammatory processes. As an expectorant agent with effects on bacterial adhesiveness, as well as antioxidant properties, erdosteine was shown to modulate mucus production and viscosity and to increase mucociliary transport in lung tissues. Moreover, the active metabolites with sulfhydryl groups, released following first-pass metabolism, were reported to exhibit an inhibitory activity against the effects of free radicals. Its anti-

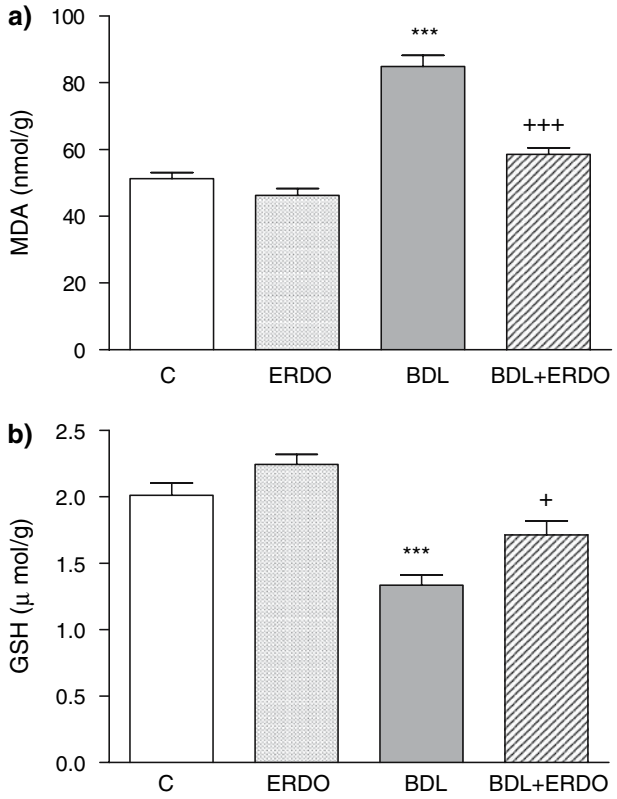

Fig. 3 **a** *MDA* Malondialdehyde and **b** *GSH* Glutathione levels of the liver tissues in the control C, *ERDO* erdosteine, *BDL* bile duct ligation and *BDL + ERDO* groups. Each group consists of ten animals. $^{***}P < 0.001$ compared with control group. $^{+}P < 0.05$, $^{+++}P < 0.001$ compared with BDL group

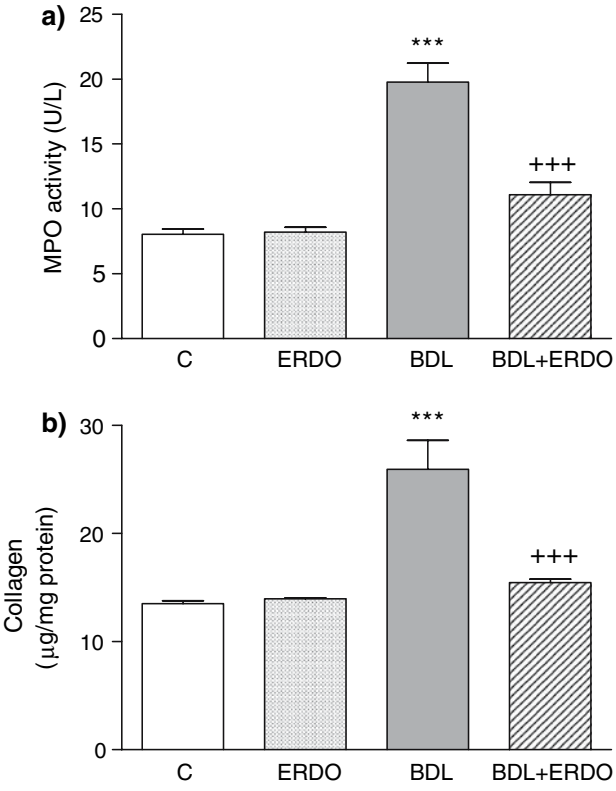

Fig. 4 **a** *MPO* Myeloperoxidase activity and **b** collagen contents of the liver tissues in the control *C*, *ERDO* erdosteine, *BDL* bile duct ligation and *BDL + ERDO* groups. Each group consists of ten animals. $^{***}P < 0.001$ compared with control group. $^{+++}P < 0.001$ compared with *BDL* group

oxidant and antifibrotic effects were also shown in bleomycin-induced lung fibrosis [14, 33]. Based on these findings, the anti-inflammatory effect of erdosteine on liver inflammation was evaluated using an experimental model of BDL in this study. Observations suggest that reactive oxygen metabolites (ROM) play a role in the recruitment of neutrophils into damaged tissue, but activated neutrophils are also a potential source of ROM [34]. Although it is not certain whether neutrophil accumulation and activation are the causes or the result of injury, increasing evidence suggests that mesengial cells and neutrophils release chemotactic substances (e.g., interleukin 8), which further promote neutrophil migration and activation [35]. Several methods have been used to define the role of neutrophils in the tissue injury. Myeloperoxidase, which is an essential enzyme for normal neutrophil function, is released as a response to various stimulatory substances [35]. In the present study, increased hepatic MPO activity due to BDL, indicates that tissue injury involves the contribution of neutrophil infiltration, and was effectively reversed by erdosteine treatment.

Bile duct ligation induces a type of liver fibrosis, which etiologically and pathogenically resembles the biliary fibrosis in the human. Injury to hepatocytes results in the generation of lipid peroxides, which may have a direct stimulatory effect on matrix production by activated stellate cells [36, 37]. Liu et al. demonstrated excessive production of superoxide radicals and hydroxyl radicals in blood and liver in rats with obstructive jaundice induced by common bile duct ligation [29]. In our study, using luminol- and lucigenin-enhanced chemiluminescence method, we quantitated the free radical generation. The two CL probes, luminol and lucigenin, differ in selectivity. Luminol detects H_2O_2, OH^-, hypochlorite, peroxynitrite, and lipid peroxyl radicals, whereas lucigenin is particularly sensitive to superoxide radical [21, 38]. In the present study, increases in luminol- and lucigenin- CL levels support the notion that hepatic injury induced by BDL involves toxic oxygen metabolites and erdosteine treatment decreases these elevations by its antioxidant action. In accordance with the increases in toxic oxygen metabolites, the hepatic MDA level was also significantly increased, indicating the presence of

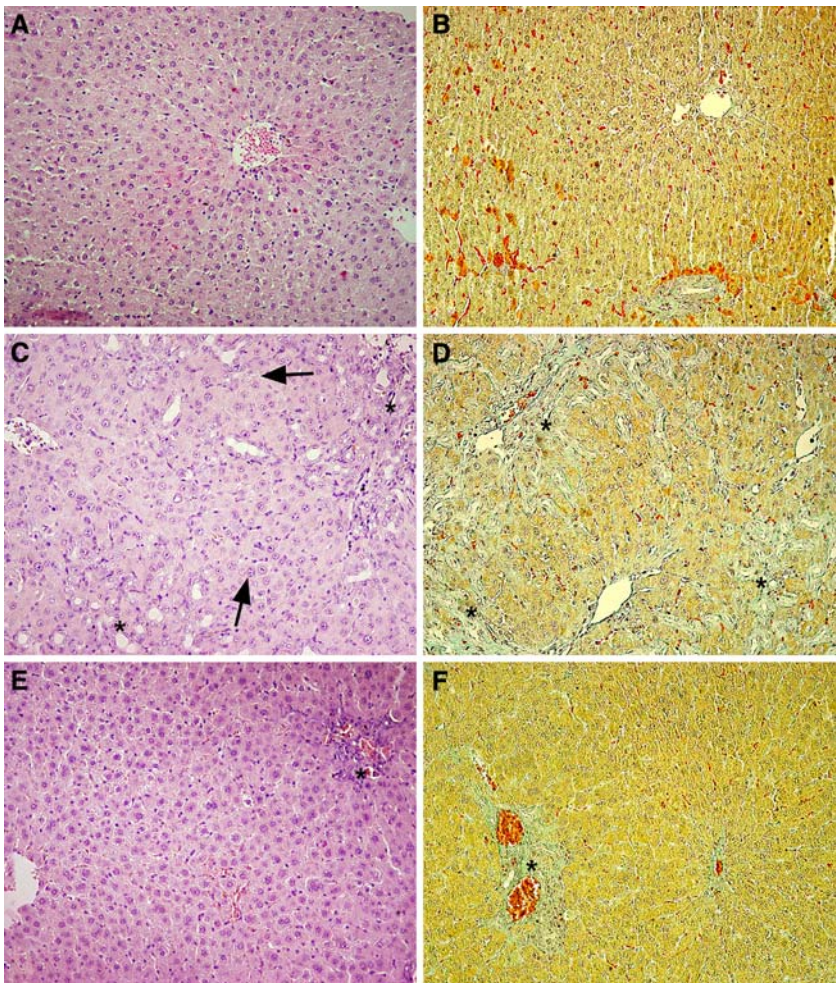

Fig. 5 Light microscopy, **a**, **b** Control group, regular liver parenchyme with hepatocytes and sinusoids. **c**, **d** BDL group, disorganization of hepatic cords, degeneration and increased eosinophilia in hepatocytes (*arrows*), fibrosis in the form of delicate bands around the acini and bile duct proliferation within the portal tract (*asterisks*). **e**, **f** BDL + ERDO group, mild disorganisation of hepatic cords, decrease in degenerated and eosinophilic hepatocytes, moderate fibrosis around the acini and decreased bile duct proliferation (*asterisks*). **a**, **c**, **e**, H&E staining; **b**, **d**, **f**, Masson's tricrome staining, original magnifications, 200×

enhanced lipid peroxidation. On the other hand, erdosteine treatment abolished the increase in malondialdehyde, probably in part by scavenging the very reactive hydroxyl, peroxyl and superoxide radicals. Erdogan et al. demonstrated that erdosteine, when administered to rats before ischemia/reperfusion of the kidney, balancing the antioxidant status, decreased the oxidant generation and lipid peroxidation [39]. Similarly, in the drug- or chemical toxicities, erdosteine was shown to suppress lipid peroxidation and reduces oxidative injury through its antioxidant effects [15, 40].

Reduced thiol agents, such as GSH, are capable of interacting with free radicals to yield more stable elements, and are known for their ability to repair membrane lipid peroxides. Ross has reported that, cell injury and enhanced cell susceptibility to toxic chemicals are related to the efflux of GSH precurcors and hence to diminished GSH biosynthesis [41]. In this sense, GSH and other antioxidants that stimulate GSH synthesis, play a critical role in limiting the propagation of free-radical reactions, which would otherwise result in extensive lipid peroxidation [42]. In the present study, accumulation of hydrophobic bile acids due to BDL leads to generation of reactive oxygen free radicals in the liver where tissue GSH stores were significantly depleted, indicating the use of GSH as an antioxidant for the detoxification of toxic oxygen metabolites [43, 44]. On the other hand, erdosteine treatment reduced the BDL-induced oxidative injury and restored the GSH levels significantly. There are several reports about the antioxidant activity of erdosteine and it was claimed that erdosteine enhances the decreased xanthine oxidase, superoxide dismutase, catalase and GSH-Px activities [14, 15, 39]. Although antioxidant enzyme activities were not determined in the present study, it may be suggested that the antioxidant effect of erdosteine may be due to the same mechanism, since tissue glutathione levels and plasma antioxidant capacity were increased following treatment. These data collectively support the hypothesis that cellular oxidative stress is a critical step in biliary obstruction-mediated injury, and suggest that antioxidant strategies designed either to scavenge free radicals, or to inhibit free radical formation may provide hepatic tissue.

Oxidative stress, in particular lipid peroxidation, induces collagen synthesis [45]. In this study, increases in the radical generation and subsequent lipid peroxidation induced an increase in fibrotic activity, as assessed by hepatic collagen content while this effect was also reduced by antioxidant erdosteine treatment. Thus, our collagen results suggest that erdosteine has an additional protective effect on oxidant-induced production and deposition of extracellular matrix components, which result in hepatic fibrosis.

In conclusion, the findings of our present study demonstrate for the first time that erdosteine, a mucoactive drug, with its potent free radical scavenging and antioxidant properties, seems to be a highly promising agent in protecting hepatic tissue against oxidative damage and in preventing hepatic fibrosis and dysfunction due to obstructive jaundice.

References

1. Poupon R, Chazouilleres O, Poupon RE (2000) Chronic cholestatic diseases. J Hepatol 32:129–140
2. Whitington PF, Balistreri WF (1991) Liver transplantation in pediatrics: indications, contraindications, and pretransplant management. J Pediatr 118:169–177
3. Qureshi WA (1999) Intrahepatic cholestatic syndromes: pathogenesis, clinical features and management. Dig Dis 17:49–59
4. Lane JR, Starbuck EM, Johnson AK, Fitts DA (1999) Effects of bile duct ligation and captopril on salt appetite and renin–aldosterone axis in rats. Physiol Behav 66:419–425
5. Kullak-Ublick GA, Meier PJ (2000) Mechanisms of cholestasis. Clin Liver Dis 4:357–385
6. Bruck R, Shirin H, Aeed H, Matas Z, Hochman A, Pines M, Avni Y (2001) Prevention of hepatic cirrhosis in rats by hydroxyl radical scavengers. J Hepatol 35:457–464
7. Zhong Z, Froh M, Lehnert M, Schoonhoven R, Yang L, Lind H, Lemasters JJ, Thurman LG (2003) Polyphenols from Camellia sinenesis attenuate experimental cholestasis-induced liver fibrosis in rats. Am J Physiol Gastrointest Liver Physiol 285:G1004–G1013
8. Montilla P, Cruz A, Padillo FJ, Tunez I, Gascon F, Munoz MC, Gomez M, Pera C (2001) Melatonin versus vitamin E as protective treatment against oxidative stress after extra-hepatic bile duct ligation in rats. J Pineal Res 31:138–144
9. Sener G, Kabasakal L, Yuksel M, Gedik N, Alican I (2005) Hepatic fibrosis in biliary-obstructed rats is prevented by Ginkgo biloba treatment. World J Gastroenterol 11:5444–5449
10. Dechant KL, Noble S (1996) Erdosteine. Drugs 52:875–881
11. Braga PC, Dal Sasso M, Zuccotti T (2000) Assessment of the antioxidant activity of the SH metabolite I of erdosteine on human neutrophil oxidative bursts. Arzneimittelforschung 50:739–746
12. Inglesi M, Nicola M, Fregnan GB, Bradamante S, Pagani G (1994) Synthesis and free radical scavenging properties of the enantiomers of erdosteine. Farmaco 40:703–708
13. Yagmurca M, Fadillioglu E, Erdogan H, Ucar M, Sogut S, Irmak MK (2003) Erdosteine prevents doxorubicin-induced cardiotoxicity in rats. Pharmacol Res 48:377–382
14. Boyaci H, Maral H, Turan G, Basyigit I, Dillioglugil MO, Yildiz F, Tugay M, Pala A, Ercin C (2006) Effects of erdosteine on bleomycin-induced lung fibrosis in rats. Mol Cell Biochem 281:129–137
15. Isik B, Bayrak R, Akcay A, Sogut S (2006) Erdosteine against acetaminophen induced renal toxicity. Mol Cell Biochem 287:185–191
16. Demiralay R, Gursan N, Erdem H (2006) The effects of erdosteine, *N*-acetylcysteine, and vitamin E on nicotine-induced apoptosis of pulmonary cells. Toxicology 219:197–207
17. Shimizu I Ma YR, Mizobuchi Y, Liu F, Miura T, Nakai Y, Yasuda M, Shiba M, Horie T, Amagaya S, Kawada N, Hori H, Ito S (1999) Effects of Sho-saiko-to, a Japanese herbal medicine, on hepatic fibrosis in rats. Hepatology 29:149–160
18. Moss DW, Henderson AR, Kachmar JF (1987) Enzymes.In: Tietz NW (ed) Fundamentals of clinical chemistry. WB Saunders, Philadelphia, pp 372–373
19. Martinek RG (1972) A rapid ultraviolent spectrophotomeetric lactic dehydrogenase assay. Clin Chem Acta 40:91–99
20. Winsten S, Cehelyk B (1969) A rapid micro diazo technique for measuring total bilirubin. Clin Chim Acta 25:441–446
21. Ohara Y, Peterson TE, Harrison DG (19939 Hypercholesterolemia increases endothelial superoxide anion production. J Clin Invest 92:2546–2551
22. Davies GR, Simmonds NJ, Stevens TR, Sheaff MT, Banatvala N, Laurenson IF, Blake DR, Rampton DS (1994) Helicobacter pylori stimulates antral mucosal reactive oxygen metabolite production in vivo. Gut 35:179–185
23. Beuge JA, Aust SD (1978) Microsomal lipid peroxidation. Meth Enzymol 52:302–11
24. Beutler E (1975) Glutathione in red blood cell metabolism. A Manuel of Biochemical Methods, Grune & Stratton, New York, pp. 112–114
25. Hillegas LM, Griswold DE, Brickson B, Albrightson-Winslow C (1990) Assesment of myeloperoxidase activity in whole rat kidney. J Pharmacol Methods 24:285–295
26. Lopez De Leon A, Rojkind M (1985) A simple micromethod for collagen and total protein determination in formalin-fixed parraffin-embedded sections. J Histochem Cytochem 33:737–743
27. Simpson KJ, Lukacs NW, Colletti L, Strieter RM, Kunkel SL (1997) Cytokines and the liver. J Hepatol 27:1120–1132
28. Tilg H, Wilmer A, Vogel W, Herold M, Nolchen B, Judmaier G, Huber C (1992) Serum levels of cytokines in chronic liver diseases. Gastroenterology 103:264–274
29. Liu TZ, Lee KT, Chern CL, Cheng JT, Stern A, Tsai LY (2001) Free radical-triggered hepatic injury of experimental obstructive jaundice of rats involves overproduction of pro-inflammatory cytokines and enhanced activation of nuclear factor kappaB. Ann Clin Lab Sci 31:383–390
30. Fernandez-Martinez E, Perez-Alvarez V, Tsutsumi V, Shibayama M, Muriel P (2006) Chronic bile duct obstruction induces changes in plasma and hepatic levels of cytokines and nitric oxide in the rat. Exp Toxicol Pathol 58:49–58
31. DeCicco LA, Rikans LE, Tutor CG, Hornbrook KR (1998) Serum and liver concentrations of tumor necrosis factor alpha and interleukin-1beta following administration of carbon tetrachloride to male rats. Toxicol Lett 98:115–121
32. Grisham MB, Granger DN (1988) Neutrophil-mediated mucosal injury. Role of reactive oxygen metabolites. Dig Dis Sci 33(3 Suppl):6S-15S
33. Sogut S, Ozyurt H, Armutcu F, Kart L, Iraz M, Akyol O, Ozen S, Kaplan S, Temel I, Yildirim Z (2004) Erdosteine prevents bleomycin-induced pulmonary fibrosis in rats. Eur J Pharmacol 494:213–220

34. Winterbourn CC, Kettle AJ (2000) Biomarkers of myeloperoxidase-derived hypochlorous acid. Free Radic Biol Med 29:403–409
35. Kettle AJ, Winterbourn CC (1997) Myeloperoxidase: a key regulator of neutrophil oxidant production. Redox Rep 3:3–15
36. Serviddio G, Pereda J, Pallardo FV, Carretero J, Borras C, Cutrin J, Vendemiale G, Poli G, Vina J, Sastre J (2004) Ursodeoxycholic acid protects against secondary biliary cirrhosis in rats by preventing mitochondrial oxidative stress. Hepatology 39:711–720
37. Lee KS, Buck M, Houglum K, Chojkier M (1995) Activation of hepatic stellate cells by TGF alpha and collagen type I is mediated by oxidative stress through c-myb expression. J Clin Invest 96:2461–2468
38. Haklar U, Yuksel M, Velioglu A, Turkmen M, Haklar G, Yalcin AS (2002) Oxygen radicals and nitric oxide levels in chondral or meniscal lesions or both. Clin Orthop Relat Res 403:135–142
39. Erdogan H, Fadillioglu E, Yagmurca M, Ucar M, Irmak MK (2006) Protein oxidation and lipid peroxidation after renal ischemia-reperfusion injury: protective effects of erdosteine and *N*-acetylcysteine. Urol Res 34:41–46
40. Terzi A, Iraz M, Sahin S, Ilhan A, Idiz N, Fadillioglu E (2004) Protective effects of erdosteine on rotenone-induced oxidant injury in liver tissue. Toxicol Ind Health 20:141–147
41. Ross D (1988) Glutathione, free radicals and chemotherapeutic agents. Mechanisms of free-radical induced toxicity and glutathione-dependent protection. Pharmacol Ther 37:231–249
42. Ohta Y, Imai Y, Matsura T, Yamada K, Tokunaga K (2005) Successively postadministred melatonin prevents disruption of hepatic antioxidant status in rats with bile duct ligation. J Pineal Res 39:367–374
43. Sokol RJ, Devereaux MW, Khandwala R (1998) Effect of oxypurinol, a xanthine oxidase inhibitor, on hepatic injury in the bile duct-ligated rat. Pediatr Res 44:397–401
44. Orellana M, Rodrigo R, Thieleman L, Guajardo V (2000) Bile duct ligation and oxidative stress in the rat: effects in liver and kidney. Comp Biochem Physiol C Toxicol Pharmacol 126:105–111
45. Muriel P, Moreno MG (2004) Effects of silymarin and vitamins E and C on liver damage induced by prolonged biliary obstruction in the rat. Basic Clin Pharmacol Toxicol 94:99–104