Current Research in Pharmacology and Drug Discovery 1 (2020) 10–18

Contents lists available at ScienceDirect

Current Research in Pharmacology and Drug Discovery

journal homepage: www.journals.elsevier.com/current-research-in-pharmacology-and-drug-discovery

Oral administration of thiol-reducing agents mitigates gut barrier disintegrity and bacterial lipopolysaccharide translocation in a rat model of biliary obstruction

Mohammad Mehdi Ommati [a,1], Omid Farshad [b,1], Hossein Niknahad [b,c], Khadijeh Mousavi [c], Marjan Moein [c], Negar Azarpira [d], Hamidreza Mohammadi [c], Akram Jamshidzadeh [b,c,*], Reza Heidari [b,*]

[a] College of Life Sciences, Shanxi Agricultural University, Taigu, Shanxi 030801, PR China
[b] Pharmaceutical Sciences Research Center, Shiraz University of Medical Sciences, Shiraz, Iran
[c] Department of Pharmacology and Toxicology, School of Pharmacy, Shiraz University of Medical Sciences, Shiraz, Iran
[d] Transplant Research Center, Shiraz University of Medical Sciences, Shiraz, Iran

ARTICLE INFO

Keywords:
Bile acids
Cirrhosis
Endotoxemia
Glutathione
Oxidative stress

ABSTRACT

It has been well documented that cirrhosis is associated with the intestinal injury. Intestinal injury in cirrhosis could lead to bacterial lipopolysaccharide (LPS) translocation to the systemic circulation. It has been found that high plasma LPS is connected with higher morbidity and mortality in cirrhotic patients. Therefore, finding therapeutic approaches to mitigate this complication has great clinical value. Several investigations mentioned the pivotal role of oxidative stress in cirrhosis-associated intestinal injury. It has been well-known that the redox balance of enterocytes is disturbed in cirrhotic patients. In the current study, the effects of thiol-reducing agents N-acetylcysteine (NAC) (0.5 and 1% w: v) and dithiothreitol (DTT) (0.5 and 1% w: v) on biomarkers of oxidative stress, tissue histopathological alterations, and LPS translocation is investigated in a rat model of cirrhosis. Bile duct ligation (BDL) surgery was used to induce cirrhosis in male Sprague-Dawley rats. Animals (n = 48; 8 animals/group) were supplemented with NAC and DTT for 28 consecutive days. Significant changes in ileum and colon markers of oxidative stress were evident in BDL rats as judged by increased reactive oxygen species (ROS), lipid peroxidation, oxidized glutathione (GSSG), and protein carbonylation along with decreased antioxidant capacity and glutathione (GSH) content. Blunted villus, decreased villus number, and inflammation was also detected in the intestine of BDL animals. Moreover, serum LPS level was also significantly higher in BDL rats. NAC and DTT administration (0.5 and 1% w: v, gavage) significantly decreased biomarkers of oxidative stress, mitigated intestinal histopathological alterations, and restored tissue antioxidant capacity. Moreover, NAC and/or DTT significantly suppressed LPS translocation to the systemic circulation. The protective effects of thiol reducing agents in the intestine of cirrhotic rats could be attributed to the effect of these chemicals on the cellular redox environment and biomarkers of oxidative stress.

1. Introduction

Intestinal barrier integrity is a critical factor which prevents pathogens penetration into the systemic circulation. It has been well-documented that gut barrier function and integrity is significantly hampered in obstructive cholestasis as well as experimental models of cirrhosis (Fukui & Wiest, 2016). Bacterial lipopolysaccharide (LPS) translocation is one of the most deleterious consequences of impaired intestinal barrier function in cholestasis/cirrhosis (Fukui & Wiest, 2016; Nolan, 2010; Wright et al., 2007). LPS plays a pivotal role in the development of severe complications in different organs, such as the liver (Nolan, 2010). The increasing level of LPS in the systemic circulation could also seriously affect other organs through inflammatory-based responses and severe oxidative stress (Fukui & Wiest, 2016; Nolan, 2010; Wright et al., 2007).

* Corresponding author. Pharmaceutical Sciences Research Center, Shiraz University of Medical Sciences, Shiraz, Iran. Fax: +987132424127.
E-mail addresses: ajamshid@sums.ac.ir (A. Jamshidzadeh), rheidari@sums.ac.ir (R. Heidari).
[1] The authors contributed equally to this work.

https://doi.org/10.1016/j.crphar.2020.06.001
Received 30 March 2020; Received in revised form 28 May 2020; Accepted 10 June 2020

Table 1
Serum biochemical measurements in cirrhotic rats treated with N-acetyl cysteine (NAC) and dithiothreitol (DTT).

	Sham	BDL	BDL + NAC 0.5%	BDL + NAC 1%	BDL + DTT 0.5%	BDL + DTT 1%
ALT (U/l)	36 ± 9[a]	296 ± 37	134 ± 29[a]	116 ± 34[a]	153 ± 38[a]	122 ± 12[a]
LDH (U/l)	448 ± 65[a]	1123 ± 198	922 ± 162[a]	959 ± 136[a]	902 ± 126[a]	763 ± 181[a]
AST (U/l)	71 ± 12[a]	387 ± 33	250 ± 20[a]	193 ± 36[a]	227 ± 33[a]	166 ± 25[a]
γGT (U/l)	20 ± 8[a]	140 ± 40	97 ± 15	90 ± 18	106 ± 21	156 ± 27
ALP (U/l)	1103 ± 211[a]	3033 ± 255	2994 ± 439	2715 ± 262	2755 ± 404	2898 ± 200
Bilirubin (mg/dl)	0.26 ± 0.06[a]	13 ± 1.3	11 ± 2.9	10 ± 2.6	13 ± 1	13 ± 1
Bile acids (μmol/l)	17 ± 4[a]	113 ± 21	90 ± 11	107 ± 18	108 ± 11	105 ± 17

Data are shown as mean ± SD (n = 8).
[a] Indicates significantly different as compared with the BDL animals ($P < 0.001$).

Bile duct ligation (BDL) is an appropriate animal model for investigating cirrhosis and its associated complications (Heidari et al., 2019a). It has been well-documented that cirrhosis-induced liver complications, spleen damage and portal hypertension, cirrhosis-associated cholemic nephropathy, sarcopenia, and muscle wasting, as well as intestinal damage and disintegrity, have been developed in the BDL rat model of cirrhosis (Heidari et al., 2019b; Heidari et al., 2019c; Rivera-Huizar et al., 2006; Tièche et al., 2001; Bosoi et al., 2017; Ara et al., 2006). On the other hand, extrapolating animal data to human diseases is a complicated procedure. However, there are some similarities between the data acquired from BDL models to human cases. For example, it has been mentioned that oxidative stress occurs in human cases of cholestasis (Martínez-Cecilia et al., 2016). In the current study, the BDL rat model was selected to investigate the effects of thiol reducing agents on gut barrier disintegrity and lipopolysaccharide translocation to the systemic circulation.

Although the exact cellular and molecular mechanisms of gut barrier disintegrity during cirrhosis are far from clear, oxidative stress seems to play a fundamental role in this complication (Assimakopoulos et al., 2004; Wang et al., 2010; Portincasa et al., 2007). Enhanced generation of reactive oxygen species (ROS) defect in cellular antioxidant defense mechanisms, and disruption of biological targets (e.g., biomembrane lipids) has been demonstrated in the intestine during cirrhosis (Assimakopoulos et al., 2004; Wang et al., 2010; Portincasa et al., 2007). It has been found that intestinal glutathione (GSH) content is also significantly depleted in experimental models of cirrhosis (Assimakopoulos et al., 2004; Wang et al., 2010; Portincasa et al., 2007). Based on these data, the administration of antioxidants and thiol reducing agents that could increase the cellular level of GSH might prevent intestinal barrier damage in cirrhosis.

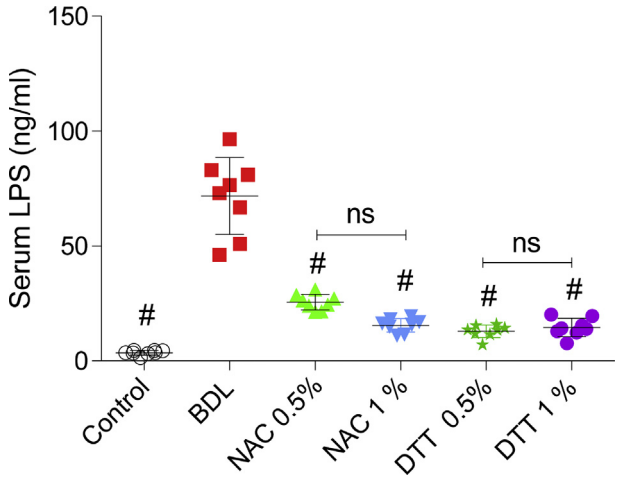

Fig. 1. Serum lipopolysaccharide (LPS) level in bile duct ligated (BDL) rats. NAC: N-acetyl cysteine; DTT: Dithiothreitol. Data are presented as mean ± SD (n = 8). # Indicates Significantly different as compared with BDL rats ($P < 0.001$). ns: not significant.

N-acetylcysteine (NAC) is a well-known antioxidant and thiol-reducing agent. NAC is clinically administered against acetaminophen hepatotoxicity. On the other hand, NAC is widely investigated for its protective properties in different experimental models (Aldini et al., 2018). NAC can replenish cellular GSH levels (Aldini et al., 2018). GSH and its associated enzymatic systems are the primary defense mechanisms against oxidative stress (Reed, 1990). On the other hand, the ability of NAC as a radical scavenger and antioxidant has also been repeatedly described (Aldini et al., 2018). Dithiothreitol (DTT) is another potent thiol reducing agent which effectively reduce the disulfide bonds in the oxidized glutathione molecule (GSSG) or other oxidized peptides and proteins (Heidari et al., 2016; Joshi et al., 2014). The ability of DTT in reducing thiol groups has been widely applied in chemical and biological studies (Heidari et al., 2016; Joshi et al., 2014; Gurunathan et al., 2013; Lopes de Almeida & Saldanha, 2010). On the other hand, it has been found that DTT could significantly protect cellular organelles against toxic insults by preserving the cellular redox environment (Joshi et al., 2014; Omidi et al., 2016; Watson et al., 2014; Deepmala et al., 2013).

As mentioned, it has been well-documented that the gut barrier is significantly damaged during cirrhosis. In the current study, NAC and DTT were administered to BDL rats as an experimental model of cirrhosis. Intestinal tissue biomarkers of oxidative stress, tissue histopathological alterations, and bacterial LPS translocation to the systemic circulation were measured.

2. Material and methods

2.1. Reagents

N-acetyl cysteine (NAC), dithiothreitol (DTT), di-nitro fluoro benzene (DNFB), trichloroacetic acid (TCA), sodium acetate, iodoacetic acid, thiobarbituric acid (TBA), dichlorofluorescein diacetate (DCFH-DA), guanidine hydrochloride, methanol HPLC grade, ethylenediamine tetra-acetic acid (EDTA), meta-phosphoric acid, acetonitrile HPLC grade, ferric chloride hexahydrate, n-propanol, 2, 4, 6-tripyridyl-s-triazine (TPTZ), reduced glutathione (GSH), potassium chloride (KCl), 2-amino-2-hydroxymethyl-propane-1,3-diol-Hydrochloride (Tris–HCl), oxidized glutathione (GSSG), dinitrophenyl hydrazine (DNPH), and glacial acetic acid were obtained from Merck (Darmstadt, Germany). Serum bile acids assay kit (EnzyFluo™ bile acid assay kit) was prepared from BioAssay Systems (USA). Kits for evaluating biomarkers of liver injury were obtained from Pars Azmoon® (Tehran, Iran). Rat Lipopolysaccharide ELISA Kit was obtained from MyBioSource® Company (California, San Diego, USA).

2.2. Animals

Male inbred Sprague-Dawley (SD) rats (7–8 weeks old, 200–300 g weight, n = 48) were obtained from Shiraz University of Medical Sciences, Shiraz, Iran. Animals were housed under a standard environment (temperature of 23±1 °C, a 12 L: 12D photoschedule, and ≈40% of relative humidity). Rats had free access to a regular pellet chow diet (RoyanFeed®, Isfahan, Iran) and tap water. All the experiments were

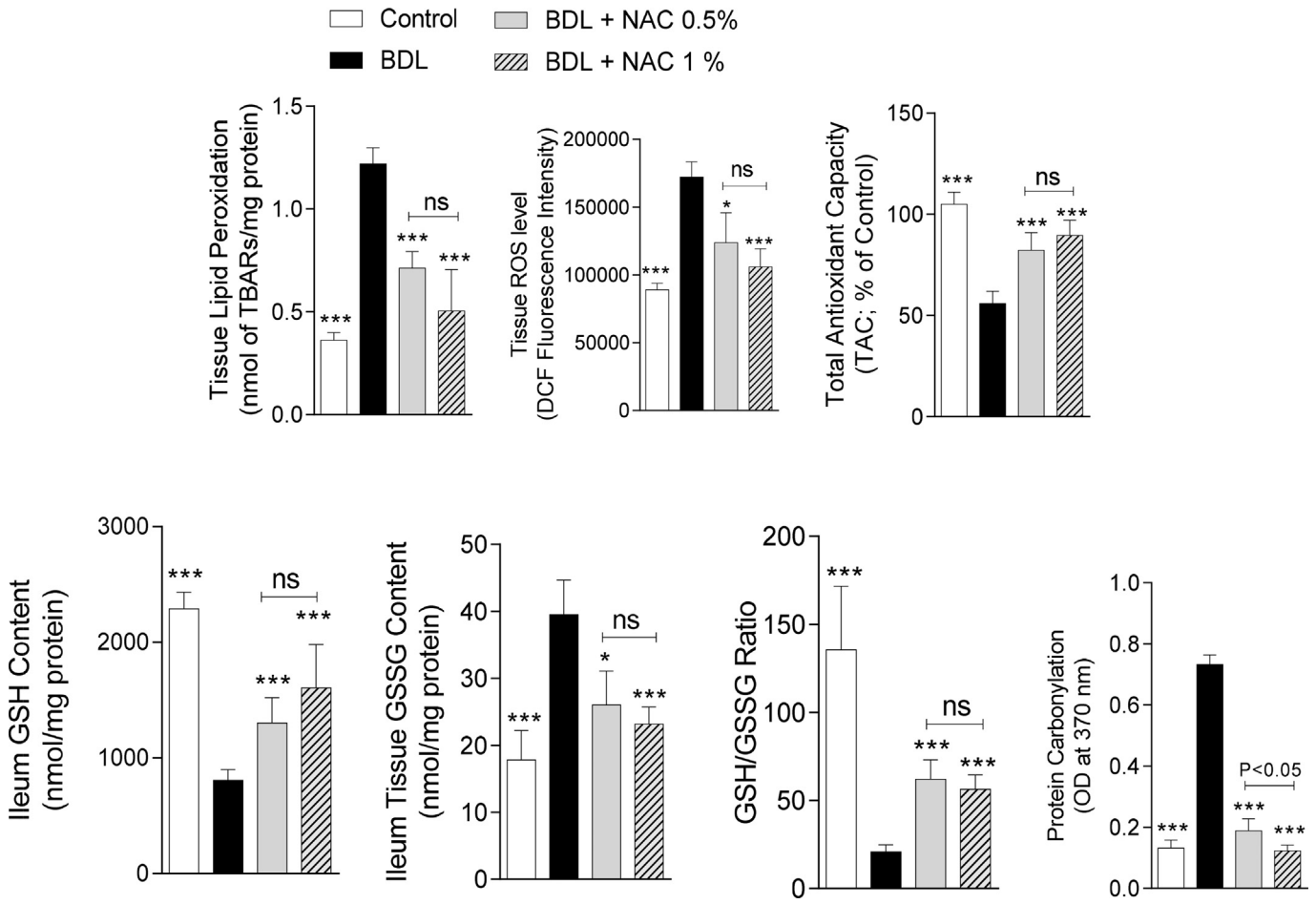

Fig. 2. Biomarkers of oxidative stress in the ileum of bile duct ligated (BDL) rats. NAC: N-acetyl cysteine. Data are presented as mean ± SD (n = 8). * & *** Indicate significantly different as compared with the BDL group ($P < 0.05$ and $P < 0.001$, respectively). ns: not significant.

performed in conformity with the guidelines for care and use of experimental animals approved by the institutional ethics committee at Shiraz University of Medical Sciences, Shiraz, Iran (# 1396-01-36-16584). Moreover, the study was performed in agreement with the current ARRIVE guidelines (Kilkenny et al., 2010).

2.3. Animal model of biliary obstruction and cirrhosis

Bile duct ligation (BDL) in rats is a reliable animal model with all complications of cholestasis/cirrhosis (Heidari et al., 2019a; Heidari et al., 2019b). Briefly, animals were anesthetized using a mixture of 10 mg/kg of xylazine and 80 mg/kg of ketamine (i.p), and a midline incision was made through the *linea alba*. Then, the common bile duct was identified, doubly ligated, and cut between these two ligatures (Heidari et al., 2019b; Moezi et al., 2013). The sham operation consisted of laparotomy and bile duct identification without ligation (Heidari et al., 2019b; Moezi et al., 2013). Four groups of BDL rats (8 rats/group) received DTT (1 mL of 0.5 and 1% w: v solution, gavage) and NAC (1 mL of 0.5 and 1% w: v solution, gavage) for 28 consecutive days after BDL operation. A BDL group (n = 8) and sham-operated rats (n = 8) received daily gavage of an equivalent volume of tap water (1 mL) as NAC and DTT solvent. On day 29, rats were deeply anesthetized (thiopental 80 mg/kg, i. p), and tissue and blood samples were collected for further studies (Kountouras et al., 1984).

2.4. Serum biochemistry, sample collection, and tissue histopathology

A MindrayBS-200® auto analyzer and commercial kits (Pars Azmoon®) were used to measure serum alanine aminotransferase (ALT), lactate dehydrogenase (LDH), alkaline phosphatase (ALP), γ-Glutamyl transferase (γ-GT), aspartate aminotransferase (AST), and bilirubin. Rat serum lipopolysaccharide (LPS) level was measured using a commercial kit (Rat Lipopolysaccharide ELISA Kit; Catalog Number. MBS704575; MyBiosource Company; California, San Diego, USA) based on the manufacturer instructions. Serum bile acids level was measured using a fluorescence-based bile acid assay kit (EnzyFluo™, BioAssay Systems, USA). Samples of small intestine and colon were rinsed with ≈10 mL of ice-cold saline (sodium chloride 0.9%, 4 °C). For histopathological assessments, tissue samples were fixed in 10% neutral buffered formalin and then embedded in paraffin. Tissue sections (5 μm thickness) were stained with hematoxylin-eosin (H&E). Quantitative analysis was then performed to assess intestinal tissue histopathological alterations in cirrhotic animals (Beutheu et al., 2014).

2.5. Reactive oxygen species (ROS) in intestinal tissue

Dichlorofluorescein diacetate (DCFH-DA) was used as a fluorescent probe to assess ROS levels in intestine specimens (Heidari et al., 2018a). Briefly, 10 μL of DCFH-DA (final concentration, 10 μM) was added to the tissue homogenate (100 μL of the tissue homogenate in 1900 μL of Tris–HCl buffer) and then incubated in the dark (10 min, 37 °C) (Heidari et al., 2019b; Heidari et al., 2019c). Finally, the fluorescence intensity of DCF was measured using a fluorimeter (FLUOstar Omega®, $\lambda_{excit} = 485$ nm, and $\lambda_{em} = 525$ nm) (Ahmadian et al., 2017a).

2.6. Ileum and colon levels of reduced and oxidized glutathione

Ileum and colon oxidized (GSSG) and reduced (GSH) glutathione levels were measured using an HPLC method (Truong et al., 2006; Meeks

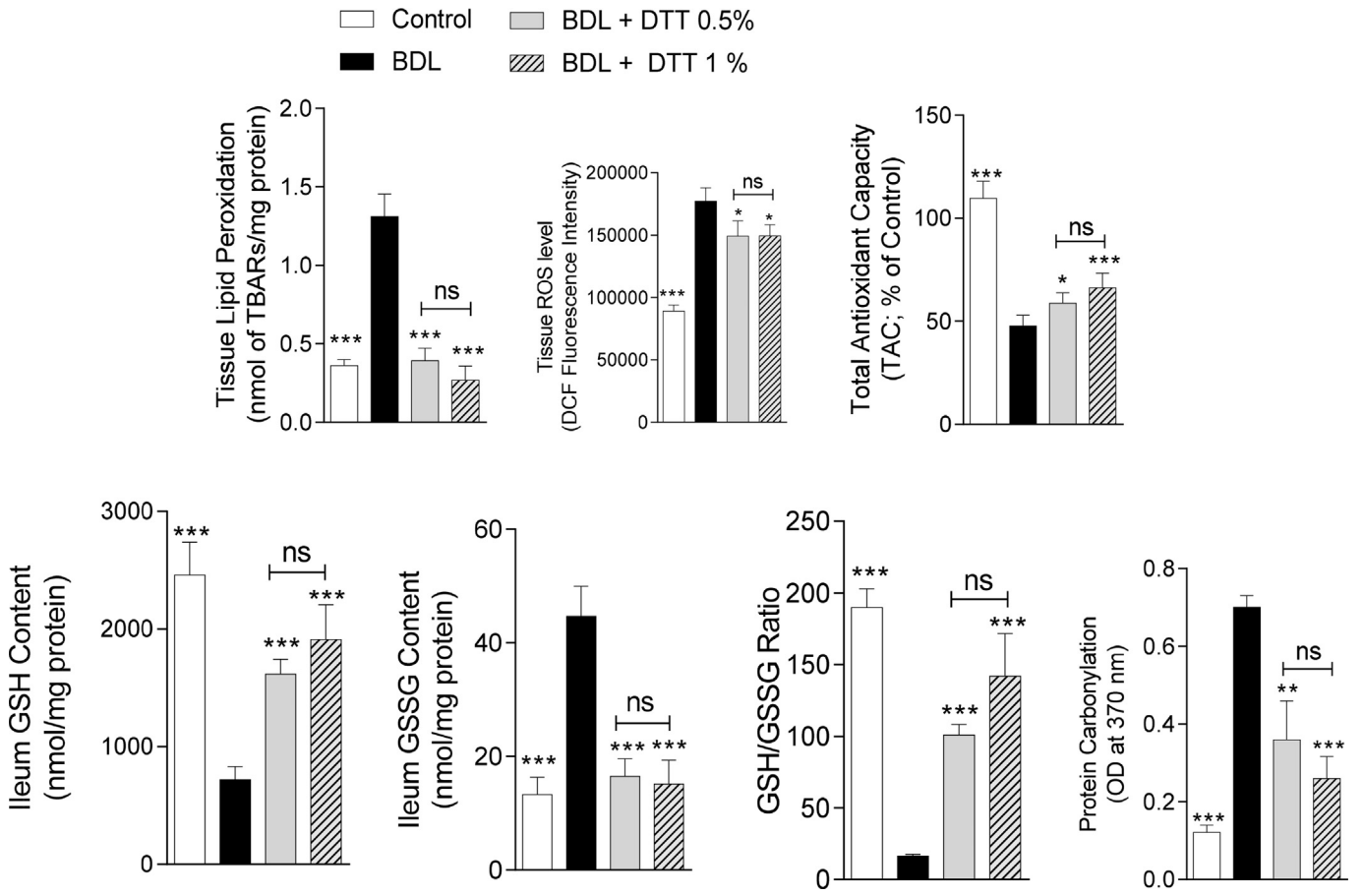

Fig. 3. Effect of dithiothreitol (DTT) administration on biomarkers of oxidative stress in the ileum of bile duct ligated (BDL) rats. Data are represented as mean ± SD (n = 6). * & *** Indicate significantly different as compared with the BDL group (P < 0.05 and P < 0.001, respectively). ns: not significant.

& Harrison, 1991). The HPLC system composed of an NH_2 column as the stationary phase (25 cm, Bischoff chromatography, Leonberg, Germany). The mobile phases included buffer A (Water: Methanol; 1: 4 v: v) and buffer B (Acetate buffer: Buffer A; 1:4 v: v) and a gradient method with a steady increase of buffer B to 95% in 25 min with a flow rate of 1 mL/min (Meeks & Harrison, 1991). For extraction of GSH and GSSG from the intestinal tissue, samples (200 mg) were homogenized in 2 mL of ice-cooled 250 mM Tris–HCl buffer (pH = 7.4; 4 °C). Then, 500 μL of meta-phosphoric acid (50% w: v) was added to deproteinize samples. Samples were mixed well, incubated on ice (10 min, 4 °C), and centrifuged (17,000 g, 30 min, 4 °C). Then, the supernatant was collected in 5 mL tubes and treated with ≈300 μL of NaOH: $NaHCO_3$ solution (2 M: 2 M) (Meeks & Harrison, 1991). Then, 100 μL of iodoacetic acid (1.5% w: v solution in deionized water) was added, and samples were incubated in the dark for 1 h (4 °C). Afterward, 500 μL of 2, 4-dinitrofluorobenzene (DNFB; 1.5% w: v solution in absolute ethanol) was added and mixed well. Samples were incubated in the dark (25 °C, 24 h). Finally, samples were centrifuged (17,000 g, 30 min, 4 °C) and 25 μL of the supernatant was injected into the aforementioned HPLC system (Truong et al., 2006; Meeks & Harrison, 1991). The UV detector was set at λ = 252 nm.

2.7. Protein carbonylation

The formation of protein carbonyl groups was assessed based on the reaction of carbonyl groups with DNPH (Levine et al., 1990). For this purpose, tissue homogenate (1 mL of 10% w: v in KCl) was treated with Triton X-100 (100 μL of 0.1%. v: v) and centrifuged (700 g, 10 min). Afterward, 500 μL aliquot of the supernatant was treated with 300 μL of 10 mM DNPH (dissolved in HCl). Samples were then incubated in the dark for 1 h (25 °C, with vortexing every 10 min). Then, 100 μL of trichloroacetic acid (20% w: v) was added, mixed well, and centrifuged (10,000 g, 5 min). The supernatant discarded, and the pellet was washed three times with 1 mL ethanol: ethyl acetate (1:1 v: v). The precipitate was re-dissolved in 600 μL of 6 M guanidine chloride (pH = 2.3) and centrifuged (12,000 g, 5 min). Finally, the absorbance of the supernatant was measured at λ = 370 nm (EPOCH® plate reader, Highland Park, USA) (Zhang et al., 2004; Heidari et al., 2014a).

2.8. Lipid peroxidation

Thiobarbituric acid reactive substances (TBARS) were measured as an index of lipid peroxidation in the intestinal tissue of cirrhotic animals (Heidari et al., 2018a; Heidari et al., 2014b; Ahmadian et al., 2017b). The TBARS assay reaction mixture composed of 500 μL of tissue homogenate (10% w: v in KCl, 1.15% w: v), 1 mL of thiobarbituric acid (0.375%, w: v), and 3 mL of phosphoric acid (1% w: v, pH = 2). Samples were mixed well and heated in a water bath (100 °C, 45 min). Then, the mixture was cooled, and then 2 mL of n-butanol was added. Samples were vortexed (30 s), and centrifuged (10,000g for 5 min). Finally, the absorbance of the upper phase (n-butanol phase) was read at λ = 532 nm (EPOCH plate reader, BioTek® instruments, Highland Park, USA) (Heidari et al., 2018a; Heidari et al., 2014b).

2.9. The ferric reducing antioxidant power (FRAP) of intestinal tissue

The FRAP assay is a method to measure the formation of ferrous (Fe^{2+})-TPTZ complex from oxidized ferric (Fe^{3+}) ion, which forms by the action of tissue electron-donating antioxidants (Heidari et al., 2019a; Heidari et al., 2019b). The working FRAP solution was freshly prepared by mixing 25 mL of acetate buffer (300 mmol/L, pH = 3.6),

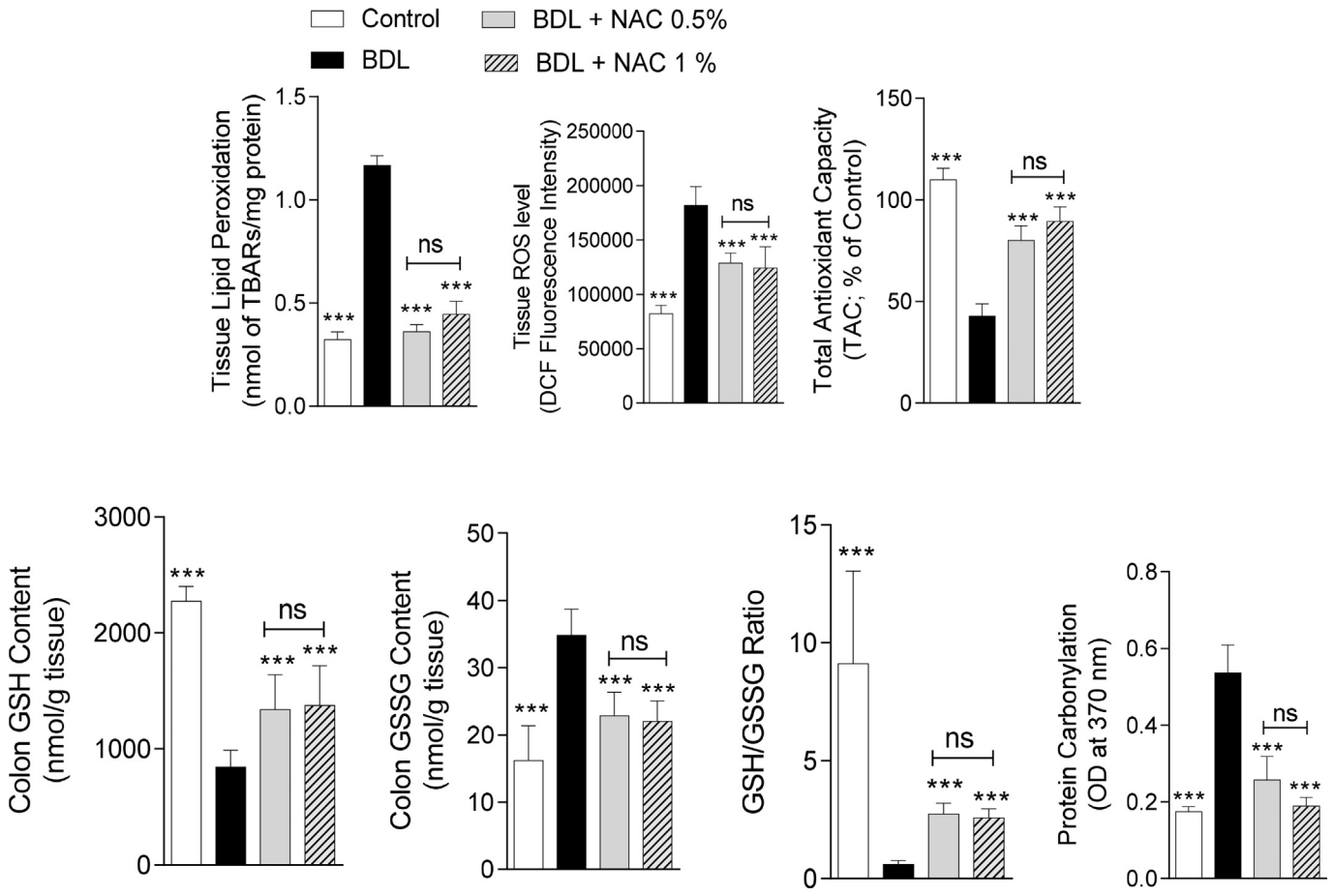

Fig. 4. Effect of N-acetyl cysteine (NAC) supplementation on the biomarkers of oxidative stress in the colon of bile duct ligated (BDL) rats. Data are given as mean ± SD (n = 6). ***Indicates significantly different as compared with the BDL group ($P < 0.001$). ns: not significant.

with 2.5 mL of ferric chloride ($FeCl_3$, 20 mmol/L), and 2.5 mL TPTZ (10 mmol/L; in 40 mmol/L HCl). Then, 100 μL of the tissue homogenate (10% w: v in Tris–HCl Buffer) was added to 900 μL of the aforementioned FRAP working solution. The mixture was incubated in the dark (37 °C, 5 min). Finally, samples were centrifuged (17,000 g, 2 min), and the absorbance of developed color was assessed at λ = 593 nm (EPOCH® plate reader, Highland Park, USA) (Alía et al., 2003).

2.10. Statistical methods

Data are represented as mean ± SD. The comparison of data sets was performed by the one-way analysis of variance (ANOVA) with Tukey's multiple comparisons as a *post hoc* test. Values of $P < 0.05$ were considered statistically significant.

3. Results

Biomarkers of liver tissue and bile duct injury were significantly increased in the serum of the BDL animals (Table 1). These data indicate the induction of liver injury and cirrhosis in the current BDL model. On the other hand, it was found that NAC (0.5 and 1%) and DTT (0.5 and 1%) treatment significantly decreased biomarkers of liver injury in cirrhotic rats (Table 1).

A significant elevation in serum LPS level was detected in cirrhotic rats (Fig. 1). It was found that NAC and DTT supplementation significantly decreased LPS translocation to the systemic circulation in the BDL animal model of cirrhosis (Fig. 1). The effect of NAC and DTT on serum LPS was not dose-dependent (Fig. 1). Moreover, no significant difference was detected between the effects of NAC and DTT on serum LPS levels in the current study (Fig. 1).

Biomarkers of oxidative stress were evaluated in the ileum and colon of cirrhotic animals (Figs. 2 and 3). Significant ROS formation, protein carbonylation, lipid peroxidation, and increased level of oxidized glutathione (GSSG) were detected in the ileum and colon tissue of the BDL group (Figs. 2 and 3). Moreover, ileum and colon GSH content were significantly decreased, and tissue antioxidant capacity was hampered in BDL animals (Figs. 2 and 3). It was found that oral administration of thiol reducing agents significantly mitigated biomarkers of oxidative stress in the ileum and colon of cirrhotic rats (Figs. 2 and 3). The effects of NAC and DTT on tissue biomarkers of oxidative stress was not dose-dependent in the current study (Figs. 2 and 3).

Ileum and colon histopathological changes in the BDL group involved blunted villus, decreased villus count, decreased crypt numbers and depth in the colon, and inflammatory response (Figs. 4 and 5). It was found that NAC (0.5 and 1%) and DTT (0.5 and 1%) treatment significantly alleviated intestinal histopathological alterations in cirrhotic animals (Figs. 4 and 5).

4. Discussion

Cirrhosis-associated enteropathy is a serious clinical complication (Tsiaoussis et al., 2015; Pijls et al., 2013). Intestinal injury during cirrhosis could lead to the translocation of bacteria and endotoxin (lipopolysaccharide; LPS) to the systemic circulation. It has been found that a higher plasma level of LPS is associated with high mortality in cirrhotic patients (Tsiaoussis et al., 2015; Arvaniti et al., 2010; Tandon & Garcia-Tsao, 2008). A high rate of death (48.7%) has been reported in

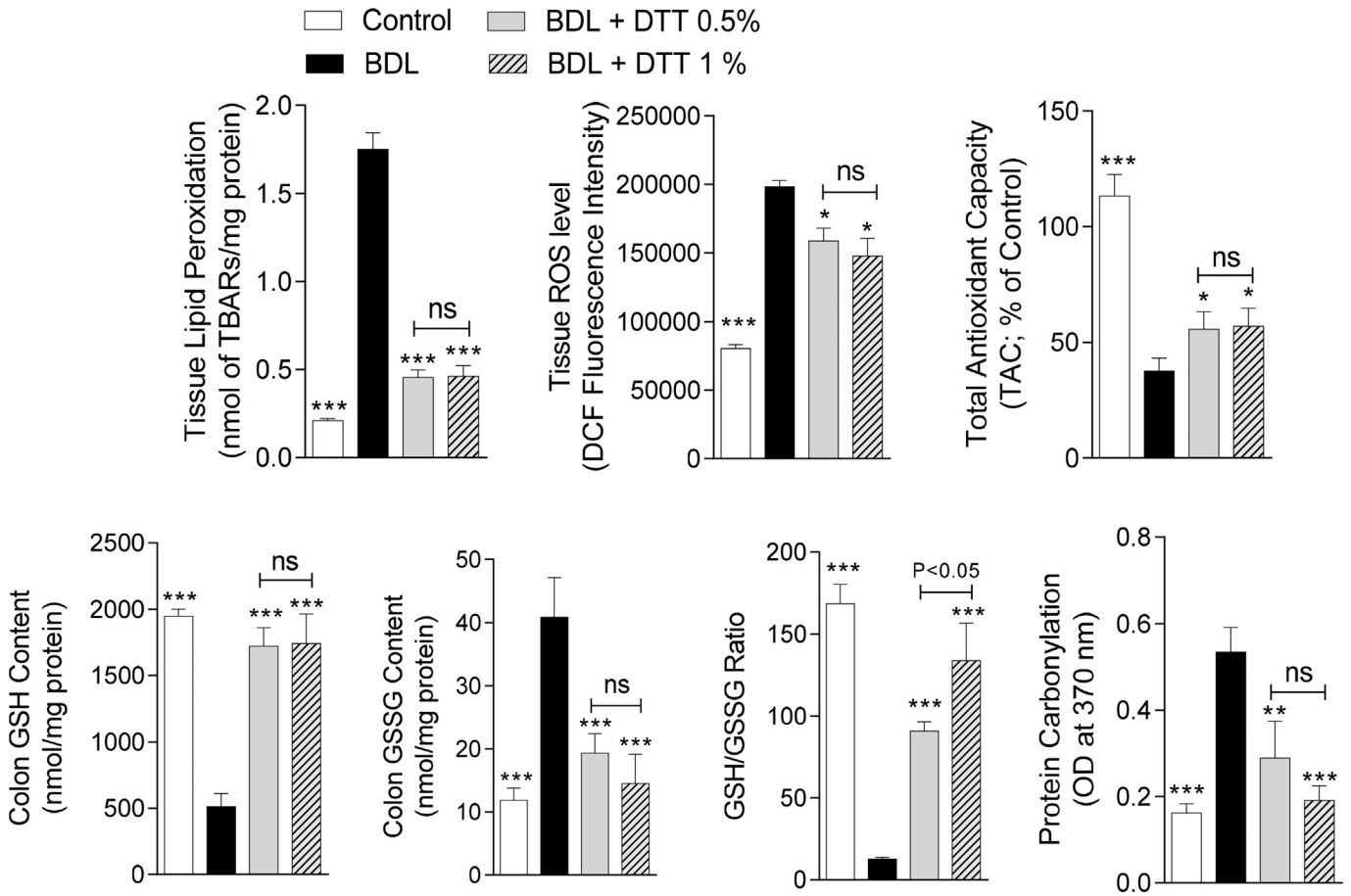

Fig. 5. Effect of dithiothreitol (DTT) supplementation on the biomarkers of oxidative stress in the colon of bile duct ligated (BDL) rats. Data are given as mean ± SD (n = 6). *, ** & *** Indicate significantly different as compared with the BDL group ($P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively). ns: not significant.

cirrhotic patients with a positive serum endotoxin test (Tarao et al., 1977). LPS is able to induce a robust systemic inflammatory response. The progression of renal disturbances, hemodynamic alterations, cirrhotic cardiomyopathy, and hepatic encephalopathy are also related to high plasma LPS levels (Fukui & Wiest, 2016; Tandon & Garcia-Tsao, 2008; Tarao et al., 1977; Parks et al., 2000). Therefore, finding therapeutic options against bacterial LPS translocation in cirrhosis could have significant clinical value.

The precise mechanisms involved in cirrhosis-associated intestinal villous atrophy and gut barrier permeability are not fully understood. However, several studies mentioned the pivotal role of oxidative stress in gut barrier injury during cholestasis/cirrhosis (Portincasa et al., 2007; Assimakopoulos et al., 2005; Giacometti et al., 2006; Wang et al., 2010; Chiva et al., 2003; Assimakopoulos et al., 2015). Increased tissue level of ROS, disturbed thiol redox state, and damaged lipids and proteins are documented in the intestinal tissue of cirrhosis models (Portincasa et al., 2007; Pijls et al., 2013; Assimakopoulos et al., 2005; Giacometti et al., 2006; Wang et al., 2010). In the current BDL model of obstructive jaundice, a significant increase in ROS level, depletion of antioxidant capacity, and damage of lipids and proteins were evident in the ileum and colon tissues. These data are in line with previous studies indicating the occurrence of oxidative stress in the intestine during cholestasis/cirrhosis (Portincasa et al., 2007; Assimakopoulos et al., 2005; Giacometti et al., 2006; Wang et al., 2010).

On the other hand, previous investigations that assessed intestinal tissue damage in cholestatic/cirrhotic models mainly focused on different parts of the small intestine (Fukui & Wiest, 2016; Assimakopoulos et al., 2004; Wang et al., 2010). In the current study, we evaluated both colon and ileum biomarkers of oxidative stress and histopathological alterations in cirrhotic animals. The microbiome flora and blood supply of colon are different from the small intestine. A high load of bacteria is found in the colon segment. The data obtained from the current study revealed the increased level of biomarkers of oxidative stress and histopathological alterations in the colon tissue of cirrhotic rats. Therefore, colon tissue injury could be a significant issue related to bacterial and endotoxin translocation during cirrhosis.

A wide range of pharmacological actions has been found for thiol reducing agents (Joshi et al., 2014; Lopes de Almeida & Saldanha, 2010; Omidi et al., 2016; Deepmala et al., 2013; Niknahad et al., 2017; Najafi et al., 2017; Heidari et al., 2015; Tee et al., 1986; Konigsberg, 1972; Cleland, 1964). These compounds can directly scavenge reactive species (Lopes de Almeida & Saldanha, 2010; Najafi et al., 2017; Heidari et al., 2015; Tee et al., 1986). On the other hand, the effects of NAC and DTT on cellular antioxidant defense mechanisms has been repeatedly reported (Aldini et al., 2018; Heidari et al., 2018b). The data obtained from the current BDL model mentioned the antioxidative effects and regulation of cellular thiol redox state as fundamental mechanisms involved in the protective effects of thiol reducing agents in the ileum and colon of cirrhotic animals. Previous studies also mentioned the potential therapeutic role of antioxidant molecules against cirrhosis-induced intestinal injury (Portincasa et al., 2007; Assimakopoulos et al., 2005; Giacometti et al., 2006; Wang et al., 2010). The administration of these antioxidants could inhibit ROS-associated signaling pathways, cell death, and tissue injury (Portincasa et al., 2007; Assimakopoulos et al., 2005; Giacometti et al., 2006; Wang et al., 2010). It has been found that severe oxidative stress could suppress cell growth (Zhang et al., 2003). This mechanism might be connected to the suppressed proliferation of enterocytes during cirrhosis. Decreased enterocytes proliferation could lead to a significant

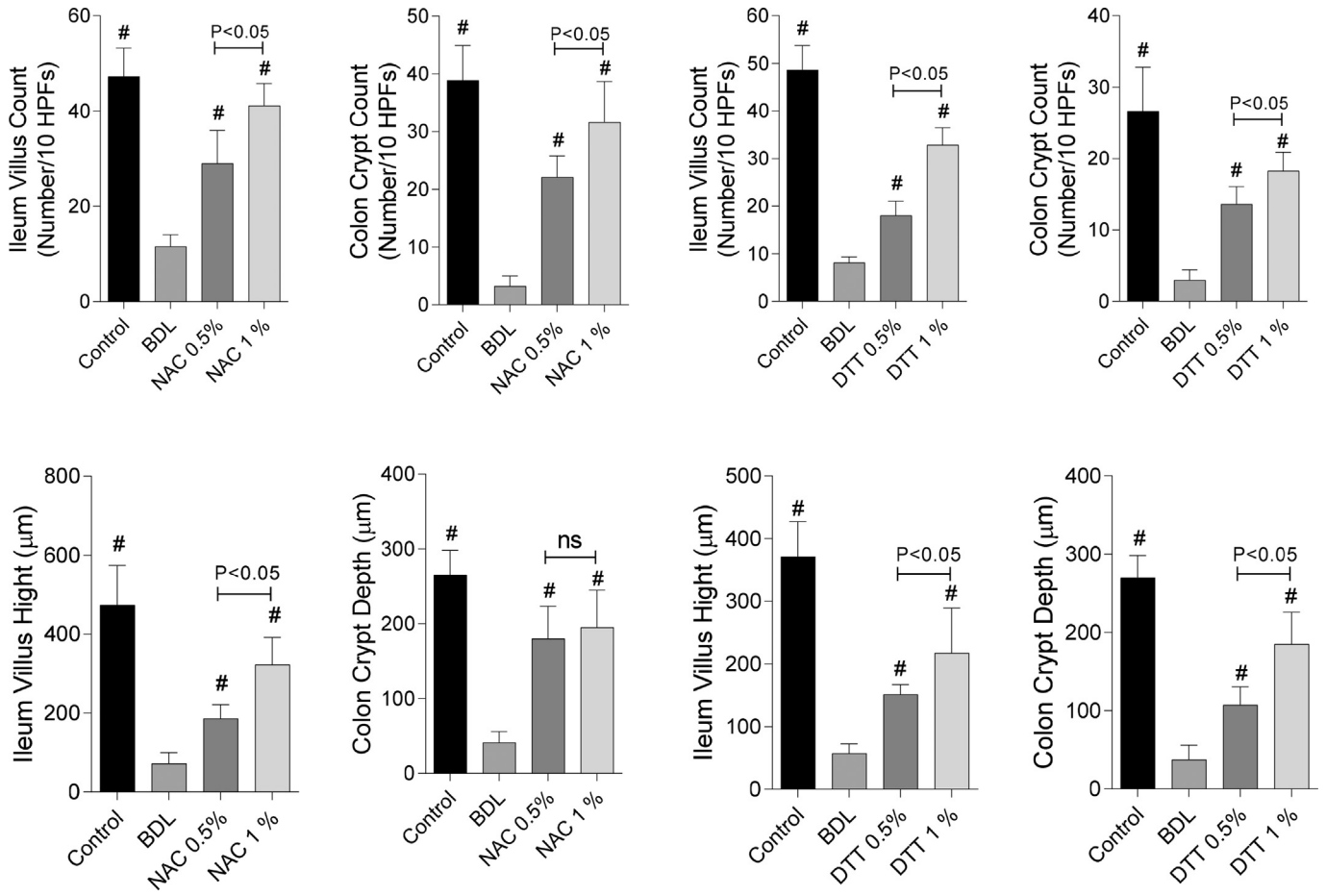

Fig. 6. Ileum and colon tissue histopathological alterations in the bile duct ligation (BDL) model of cirrhosis. NAC: N-acetyl cysteine; DTT: Dithiothreitol. Data are given as mean ± SD (n = 6). # Indicates significantly different as compared with the BDL group ($P < 0.001$). ns: not significant.

decrease in height and number of villus in ileum as well as decreased crypt depth in the colon of cirrhotic animals (Figs. 7 and 8). It has been found that the administration of antioxidants could prevent ROS-induced cell proliferation arrest (Zhang et al., 2003). In the current study, the higher villus number and height in the ileum, as well as increased colon crypt depth in thiol-reducing agents-treated cirrhotic animals, might be connected to the effects of these compounds on the intestinal cell proliferation.

Previously, we found that thiol reducing agents such as DTT and NAC could effectively protect kidney, brain, and liver tissue in animal models

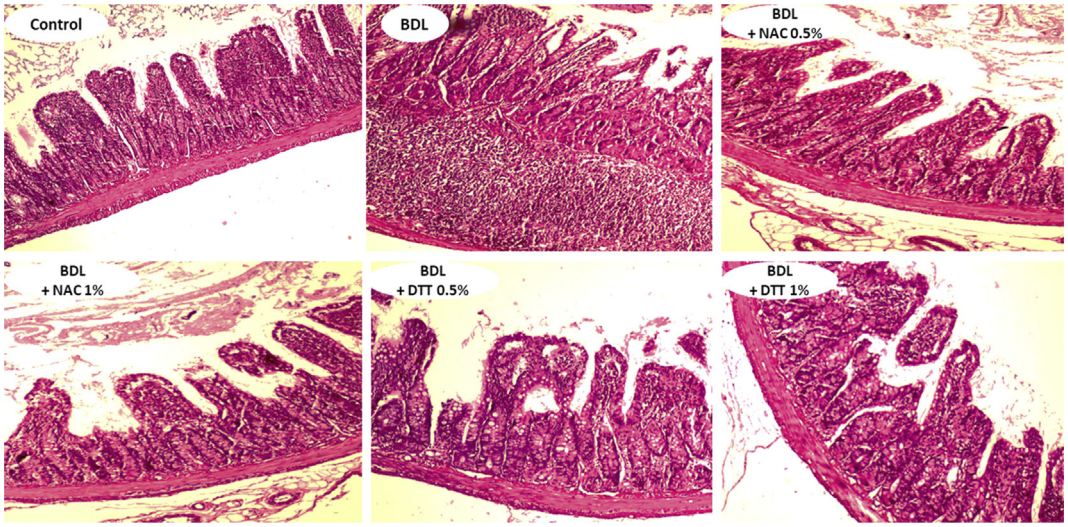

Fig. 7. Ileum tissue histopathological alterations in bile duct ligated (BDL) rats. Significant inflammation decreased villus number, and a blunted villus was detected in the ileum of BDL animals (28 days after BDL surgery). DTT and NAC administration mitigated BDL-induced ileum histopathological alterations. The grade of tissue histopathological alterations is given in Fig. 6.

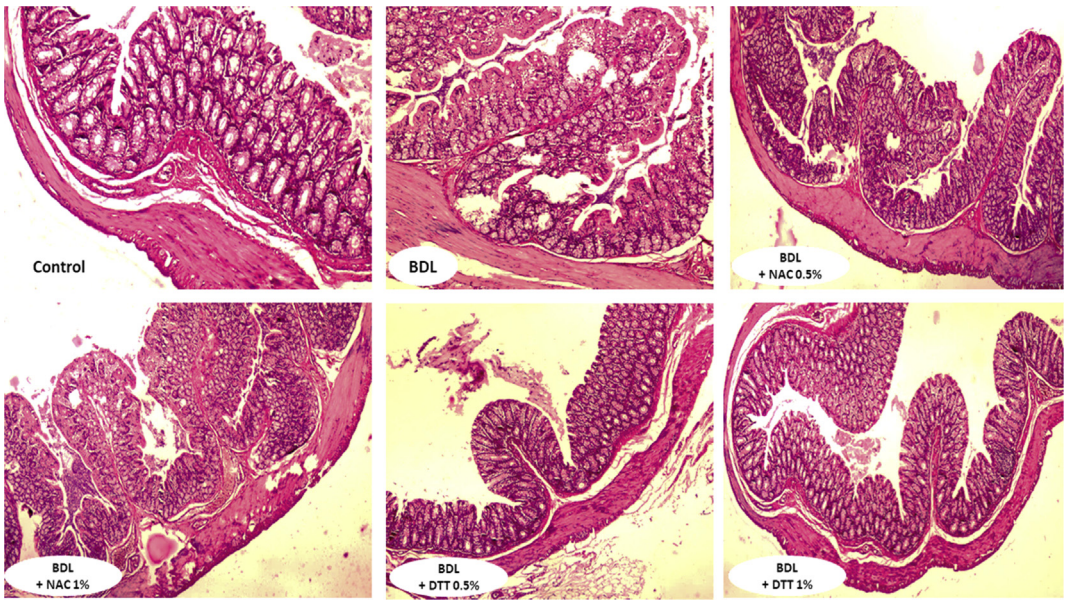

Fig. 8. Photomicrographs of the colon in bile duct ligated (BDL) rat model of cholestasis. Colon tissue histopathological changes in BDL rats included blunted villus and decreased villus numbers. It was found that DTT and NAC supplementation decreased cirrhosis-associated histopathological alterations in the colon of BDL animals. The grades of tissue histopathological alterations are represented in Fig. 6.

of acute and chronic hepatic encephalopathy and cirrhosis (Heidari et al., 2018b; Ommati et al., 2017). The data obtained from this study revealed that these compounds are also able to preserve intestinal tissue integrity and prevent bacterial LPS translocation in cirrhotic animals. Thiol-reducing agents such as NAC are FDA approved drugs for use in clinical situations (e.g., acetaminophen poisoning). Hence, these compounds could readily undergo clinical trials for the treatment of cirrhosis-associated disorders such as intestinal injury and permeability. The difference in the composition of rats and humans' bile constituents might serve as a limitation for the extrapolation of animal data to the clinical situation. However, the BDL model has been repeatedly mentioned as an appropriate model for investigating intestinal injury and bacterial LPS translocation to the systemic circulation (Bedirli et al., 2009; Lorenzo-Zuñiga et al., 2006; Kanter et al., 2016). The occurrence of oxidative stress in the intestinal tissue of human cases of cholestasis/cirrhosis also has been mentioned in previous studies (Tsiaoussis et al., 2015; Assimakopoulos et al., 2015). Therefore, the same results could be obtained in human cases of cirrhosis-associated intestinal barrier disintegrity.

The data obtained from this study suggest that the administration of NAC and DTT could be an effective strategy for the management of complications associated with endotoxemia in cirrhotic patients. On the other hand, thiol-reducing agents such as NAC and DTT might also be useful in other clinical situations such as surgery trauma and alcoholic liver disease, which are associated with a leaky gut. Further investigations are warranted to reveal the clinical significance of these data.

Author contributions

Mohammad Mehdi Ommati, Omid Farshad, Marjan Moein, Khadijeh Mousavi, Hamidreza Mohammadi, and Reza Heidari were involved in data collection and experimental setup. Professor Hossein Niknahad, and Professor Akram Jamshidzadeh were involved in study concept and manuscript draft preparation and revision. Reza Heidari and Mohammad Mehdi Ommati performed statistical analysis. Professor Negar Azarpira carried out histopathological evaluations. All authors read and approved the draft(s) as well as the final version of the manuscript.

Declaration of competing interest

The authors declare no conflicts of interest.

Acknowledgments

The authors gratefully acknowledge the Vice Chancellor for Research Affairs and Pharmaceutical Sciences Research Center, Shiraz University of Medical Sciences, and Shanxi Agricultural University, Shanxi Government Scholarship for International Research Assistant for financial (Grants # 1396-01-36-16584 and No. 2018YJ33, respectively) and technical support of this investigation.

References

Ahmadian, E., Eftekhari, A., Fard, J.K., et al., 2017. In vitro and in vivo evaluation of the mechanisms of citalopram-induced hepatotoxicity. Arch Pharm. Res. 40, 1296–1313.
Ahmadian, E., Babaei, H., Mohajjel Nayebi, A., Eftekhari, A., Eghbal, M.A., 2017. Mechanistic approach for toxic effects of bupropion in primary rat hepatocytes. Drug Res. 67, 217–222.
Aldini, G., Altomare, A., Baron, G., et al., 2018. N-Acetylcysteine as an antioxidant and disulphide breaking agent: the reasons why. Free Radic. Res. 52, 751–762.
Alía, M., Horcajo, C., Bravo, L., Goya, L., 2003. Effect of grape antioxidant dietary fiber on the total antioxidant capacity and the activity of liver antioxidant enzymes in rats. Nutr. Res. 23, 1251–1267.
Ara, C., Esrefoglu, M., Polat, A., et al., 2006. The effect of caffeic acid phenethyl ester on bacterial translocation and intestinal damage in cholestatic rats. Dig. Dis. Sci. 51, 1754–1760.
Arvaniti, V., D'Amico, G., Fede, G., et al., 2010. Infections in patients with cirrhosis increase mortality four-fold and should be used in determining prognosis. Gastroenterology 139, 1246–1256, 1256.e1241-1245.
Assimakopoulos, S.F., Vagianos, C.E., Zervoudakis, G., et al., 2004. Gut regulatory peptides bombesin and neurotensin reduce hepatic oxidative stress and histological alterations in bile duct ligated rats. Regul. Pept. 120, 185–193.
Assimakopoulos, S.F., Scopa, C.D., Zervoudakis, G., et al., 2005. Bombesin and neurotensin reduce endotoxemia, intestinal oxidative stress, and apoptosis in experimental obstructive jaundice. Ann. Surg. 241, 159–167.
Assimakopoulos, S.F., Tsamandas, A.C., Tsiaoussis, G.I., et al., 2015. Intestinal mucosal proliferation, apoptosis and oxidative stress in patients with liver cirrhosis. Ann. Hepatol. 12, 301–307.
Bedirli, A., Kerem, M., Ofluoglu, E., et al., 2009. Administration of Chlorella sp. microalgae reduces endotoxemia, intestinal oxidative stress and bacterial translocation in experimental biliary obstruction. Clin. Nutr. 28, 674–678.
Beutheu, S., Ouelaa, W., Guérin, C., et al., 2014. Glutamine supplementation, but not combined glutamine and arginine supplementation, improves gut barrier

function during chemotherapy-induced intestinal mucositis in rats. Clin. Nutr. 33, 694–701.
Bosoi, C.R., Oliveira, M.M., Ochoa-Sanchez, R., et al., 2017. The bile duct ligated rat: a relevant model to study muscle mass loss in cirrhosis. Metab. Brain Dis. 32, 513–518.
Chiva, M., Guarner, C., Peralta, C., et al., 2003. Intestinal mucosal oxidative damage and bacterial translocation in cirrhotic rats. Eur. J. Gastroenterol. Hepatol. 15, 145.
Cleland, W.W., 1964. Dithiothreitol, a new protective reagent for SH groups*. Biochemistry 3, 480–482.
Deepmala, J., Deepak, M., Srivastav, S., Sangeeta, S., Kumar, S.A., Kumar, S.S., 2013. Protective effect of combined therapy with dithiothreitol, zinc and selenium protects acute mercury induced oxidative injury in rats. J. Trace Elem. Med. Biol. 27, 249–256.
Fukui, H., Wiest, R., 2016. Changes of intestinal functions in liver cirrhosis. IID 1, 24–40.
Giacometti, A., Cirioni, O., Ghiselli, R., et al., 2006. Amphibian peptides prevent endotoxemia and bacterial translocation in bile duct-ligated rats. Crit. Care Med. 34, 2415–2420.
Gurunathan, S., Han, J.W., Dayem, A.A., et al., 2013. Antibacterial activity of dithiothreitol reduced graphene oxide. J. Ind. Eng. Chem. 19, 1280–1288.
Heidari, R., Babaei, H., Eghbal, M.A., 2014. Amodiaquine-induced toxicity in isolated rat hepatocytes and the cytoprotective effects of taurine and/or N-acetyl cysteine. Res. Pharm. Sci. 9, 97–105.
Heidari, R., Babaei, H., Roshangar, L., Eghbal, M.A., 2014. Effects of enzyme induction and/or glutathione depletion on methimazole-induced hepatotoxicity in mice and the protective role of N-acetylcysteine. Adv. Pharmaceut. Bull. 4, 21–28.
Heidari, R., Taheri, V., Rahimi, H.R., Yeganeh, B.S., Niknahad, H., Najibi, A., 2015. Sulfasalazine-induced renal injury in rats and the protective role of thiol-reductants. Ren. Fail. 1–5.
Heidari, R., Esmailie, N., Azarpira, N., Najibi, A., Niknahad, H., 2016. Effect of thiol-reducing agents and antioxidants on sulfasalazine-induced hepatic injury in normotermic recirculating isolated perfused rat liver. Toxicol. Res. 32, 133–140.
Heidari, R., Ahmadi, F., Rahimi, H.R., et al., 2018. Exacerbated liver injury of antithyroid drugs in endotoxin-treated mice. Drug Chem. Toxicol. 1–9.
Heidari, R., Ghanbarinejad, V., Mohammadi, H., et al., 2018. Dithiothreitol supplementation mitigates hepatic and renal injury in bile duct ligated mice: potential application in the treatment of cholestasis-associated complications. Biomed. Pharmacother. 99, 1022–1032.
Heidari, R., Niknahad, H., 2019. The role and study of mitochondrial impairment and oxidative stress in cholestasis. In: Vinken, M. (Ed.), Experimental Cholestasis Research. Springer New York, New York, NY, pp. 117–132.
Heidari, R., Mandegani, L., Ghanbarinejad, V., et al., 2019. Mitochondrial dysfunction as a mechanism involved in the pathogenesis of cirrhosis-associated cholemic nephropathy. Biomed. Pharmacother. 109, 271–280.
Heidari, R., Mohammadi, H., Ghanbarinejad, V., et al., 2019. Proline supplementation mitigates the early stage of liver injury in bile duct ligated rats. J. Basic Clin. Physiol. Pharmacol. 30, 91–101.
Joshi, D., Mittal, D.K., Shukla, S., Srivastav, A.K., Srivastav, S.K., 2014. Methylmercury toxicity: amelioration by selenium and water-soluble chelators as N-acetyl cysteine and dithiothreitol. Cell Biochem. Funct. 32, 351–360.
Kanter, M., Takir, M., Mutlu, H.H., Kanter, B., Kostek, O., Toprak, A.E., 2016. Protective effects of curcumin on intestinal damage in cholestatic rats. J. Invest. Surg. 29, 128–136.
Kilkenny, C., Browne, W.J., Cuthill, I.C., Emerson, M., Altman, D.G., 2010. Improving bioscience Research reporting: the ARRIVE guidelines for reporting animal Research. PLoS Biol. 8, e1000412.
Konigsberg, W., 1972. [13] Reduction of disulfide bonds in proteins with dithiothreitol. Methods Enzymol. 25, 185–188.
Kountouras, J., Billing, B.H., Scheuer, P.J., 1984. Prolonged bile duct obstruction: a new experimental model for cirrhosis in the rat. Br. J. Exp. Pathol. 65.
Levine, R.L., Garland, D., Oliver, C.N., et al., 1990. Determination of carbonyl content in oxidatively modified proteins. Methods Enzymol. 186.
Lopes de Almeida, J.P., Saldanha, C., 2010. Dithiothreitol revisited in red cells: a new head for an old hat. Clin. Hemorheol. Microcirc. 46, 51–56.
Lorenzo-Zuñiga, V., Rodriguez-Ortigosa, C.M., Bartoli, R., et al., 2006. Insulin-like growth factor I improves intestinal barrier function in cirrhotic rats. Gut 55, 1306–1312.
Martínez-Cecilia, D., Reyes-Díaz, M., Ruiz-Rabelo, J., et al., 2016. Oxidative stress influence on renal dysfunction in patients with obstructive jaundice: a case and control prospective study. J. Redox Biol. 8, 160–164.
Meeks, R.G., Harrison, S., 1991. Hepatotoxicology. CRC Press, New York.
Moezi, L., Heidari, R., Amirghofran, Z., Nekooeian, A.A., Monabati, A., Dehpour, A.R., 2013. Enhanced anti-ulcer effect of pioglitazone on gastric ulcers in cirrhotic rats: the role of nitric oxide and IL-1b. Pharmacol. Rep. 65, 134–143.
Najafi, N., Jamshidzadeh, A., Fallahzadeh, H., et al., 2017. Valproic acid-induced hepatotoxicity and the protective role of thiol reductants. Trends Pharmaceut. Sci. 3, 63–70.
Niknahad, H., Hosseini, H., Gozashtegan, F., et al., 2017. The hepatoprotective role of thiol reductants against mitoxantrone-induced liver injury. Trends Pharmaceut. Sci. 3, 113–122.
Nolan, J.P., 2010. The role of intestinal endotoxin in liver injury: a long and evolving history. Hepatology 52, 1829–1835.
Omidi, M., Niknahad, H., Mohammadi-Bardbori, A., 2016. Dithiothreitol (DTT) rescues mitochondria from nitrofurantoin-induced mitotoxicity in rat. J. Biochem. Mol. Toxicol. 30, 588–592.
Ommati, M.M., Jamshidzadeh, A., Niknahad, H., et al., 2017. N-acetylcysteine treatment blunts liver failure-associated impairment of locomotor activity. PharmaNutrition 5, 141–147.
Parks, R.W., Cameron, C.H.S., Gannon, C.D., Pope, C., Diamond, T., Rowlands, B.J., 2000. Changes in gastrointestinal morphology associated with obstructive jaundice. J. Pathol. 192, 526–532.
Pijls, K.E., Jonkers, D.M.A.E., Elamin, E.E., Masclee, A.A.M., Koek, G.H., 2013. Intestinal epithelial barrier function in liver cirrhosis: an extensive review of the literature. Liver Int. 33, 1457–1469.
Portincasa, P., Grattagliano, I., Testini, M., et al., 2007. Parallel intestinal and liver injury during early cholestasis in the rat: modulation by bile salts and antioxidants. Free Radical Biol. Med. 42, 1381–1391.
Reed, D.J., 1990. Glutathione: toxicological implications. Annu. Rev. Pharmacol. Toxicol. 30, 603–631.
Rivera-Huizar, S., Rincón-Sánchez, A.R., Covarrubias-Pinedo, A., et al., 2006. Renal dysfunction as a consequence of acute liver damage by bile duct ligation in cirrhotic rats. Exp. Toxicol. Pathol. 58, 185–195.
Tandon, P., Garcia-Tsao, G., 2008. Bacterial infections, sepsis, and multiorgan failure in cirrhosis. Semin. Liver Dis. 28, 26–42.
Tarao, K., So, K., Moroi, T., Ikeuchi, T., Suyama, T., 1977. Detection of endotoxin in plasma and ascitic fluid of patients with cirrhosis: its clinical significance. Gastroenterology 73, 539–542.
Tee, L.B.G., Boobis, A.R., Huggett, A.C., Davies, D.S., 1986. Reversal of acetaminophen toxicity in isolated hamster hepatocytes by dithiothreitol. Toxicol. Appl. Pharmacol. 83, 294–314.
Tièche, S., De Gottardi, A., Kappeler, A., et al., 2001. Overexpression of endothelin-1 in bile duct ligated rats: correlation with activation of hepatic stellate cells and portal pressure. J. Hepatol. 34, 38–45.
Truong, D.H., Eghbal, M.A., Hindmarsh, W., Roth, S.H., O'Brien, P.J., 2006. Molecular mechanisms of hydrogen sulfide toxicity. Drug Metab. Rev. 38, 733–744.
Tsiaoussis, G.I., Assimakopoulos, S.F., Tsamandas, A.C., Triantos, C.K., Thomopoulos, K.C., 2015. Intestinal barrier dysfunction in cirrhosis: current concepts in pathophysiology and clinical implications. World J. Hepatol. 7, 2058–2068.
Wang, P., Gong, G., Wei, Z., Li, Y., 2010. Ethyl pyruvate prevents intestinal inflammatory response and oxidative stress in a rat model of extrahepatic cholestasis. J. Surg. Res. 160, 228–235.
Watson, S.N., Lee, J.R., Risling, T.E., Hermann, P.M., Wildering, W.C., 2014. Diminishing glutathione availability and age-associated decline in neuronal excitability. Neurobiol. Aging 35, 1074–1085.
Wright, G., Davies, N.A., Shawcross, D.L., et al., 2007. Endotoxemia produces coma and brain swelling in bile duct ligated rats. Hepatology 45, 1517–1526.
Zhang, Z., Leonard, S.S., Huang, C., Vallyathan, V., Castranova, V., Shi, X., 2003. Role of reactive oxygen species and MAPKs in vanadate-induced G(2)/M phase arrest. Free Radic. Biol. Med. 34, 1333–1342.
Zhang, Y.-T., Zheng, Q.-S., Pan, J., Zheng, R.-L., 2004. Oxidative damage of biomolecules in mouse liver induced by morphine and protected by antioxidants. Basic Clin. Pharmacol. Toxicol. 95, 53–58.