

THE HYDROALCOHOLIC EXTRACT OF *ROSMARINUS OFFICINALIS* ATTENUATES LIVER DAMAGE AFTER BILE-DUCT LIGATION IN RATS

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ABSTRACT

The aim of the present study was to investigate the possible hepatoprotective effects of hydroalcoholic extract of *Rosmarinus officinalis* (RO) against liver damage in bile duct ligation (BDL)-induced cholestatic rats. Thirty-four male Wistar rats (225± 25 g) were randomly divided into four groups; sham control (SC, N=7), BDL (N=9), BDL+ 500 mg/kg/day hydroalcoholic extract of RO (N=9), and BDL+ 50 mg/kg/day silimarin (N=9). Two weeks later, the rats were sacrificed, the blood and liver were collected for liver enzyme, plasma and hepatic oxidative stress markers, antioxidant enzymes activity and hematoxylin and eosin staining. The BDL model significantly elevated the plasma oxidative stress markers and liver enzymes, as well as decreased the liver total thiol and ferric reducing antioxidant power as compared to SC group ($p \leq 0.05$). Hydroalcoholic extract of RO and silimarin significantly reduced liver enzymes and histology indices ($p \leq 0.05$), however, they did not show a significant effect on oxidative stress markers. It was concluded that the hydroalcoholic extract of *Rosmarinus officinalis* has a hepatoprotective effect on liver damage in rats induced by BDL.

Keywords: Liver; Cholestasis; Bile duct ligated; *Rosmarinus officinalis*.

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INTRODUCTION

The liver is the main source of metabolism in our body. Thus, its role in maintaining, functioning and homeostasis of the body is significant. Chronic liver disease is a major cause of global health problems, causing around 800,000 deaths worldwide annually (Saleh *et al.*, 2015). Cholestasis is a common liver disease (Alaca *et al.*, 2017). Any condition in which the flow of bile from the liver is slowed or blocked is called cholestasis, which results in abnormal accumulation of bile acids. Extrahepatic cholestasis may be due to bile duct tumors, duct narrowing, stones in the bile ducts such as cholesterol rocks, pancreatitis, and pancreatic tumor (Zhang *et al.*, 2015, Shaker *et al.*, 2015). In contrast, intrahepatic cholestasis can occur due to alcoholic liver disease, liver abscess, tuberculosis, and viral hepatitis. Clinically, cholestasis is associated with symptoms such as fatigue, itching, jaundice, dark urine and pale stools (Shaker *et al.*, 2015). The mechanism of hepatic damage in cholestasis are multifactorial, however, remaining bile acids, oxidative stress and inflammation are important factors contributing to cholestatic damage (Pan *et al.*, 2010). Bile acids increases the oxidative modification of lipids, proteins, and nucleic acids which eventually causes apoptosis or necrosis of the liver cells. In addition,

bile acids also affect the kupffer cells to produce reactive oxygen species (ROSs), thereby increasing hepatocellular damage (Gonzalez-Sanchez *et al.*, 2016). Several studies show that ROSs play an important role in the pathogenesis of liver injury and biliary obstruction (Ara *et al.*, 2005, Terzioglu *et al.*, 2017, Montilla *et al.*, 2001, Chen *et al.*, 2009). Oxidative stress has been shown to alter the shape of the hepatic stellate cells into activated star cells that would induce fibrosis (Kabirifar *et al.*, 2017a). It is known that cells are protected by the balance between production and decomposition of ROSs against potential cytotoxicity. ROSs in normal mode play an important role in signaling and homeostasis of the cell, however, excessive production of ROSs effect lipids, proteins, carbohydrates, and nucleic acids. Oxidative stress occurs as a result of an imbalance between the oxidants (such as ROS) and antioxidants (Ihsan *et al.*, 2018). Normal cells have a number of enzymatic and nonenzymatic endogenous defense systems to protect them from oxidants. The enzymatic defense system includes superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPX). They quickly eliminate ROSs such as superoxide anion, hydrogen peroxide and hydroxyl free radicals (Lee *et al.*, 2004). The best and most common method for measuring free radicals and oxidative stress is to determine the products

of the reaction of free radicals with biomolecules such as lipids, proteins, and nucleic acids (Salmaninejad *et al.*, 2017).

Medicinal plants have active biological compounds that can be used as alternatives or complement conventional drugs with a wide range of medicinal properties (Wang *et al.*, 2017). *Rosmarinus officinalis* (RO) is a Mediterranean herb that grows in the North Africa, South-Europe and the United States, but today it is cultivated in different parts of the world due to its aromatic properties (Rašković *et al.*, 2014). This plant is from family of lamiaceae and contains at least 1% volatile oil. In traditional medicine, this plant is used to treat headaches, migraines, blood pressure, liver problems, and physical and mental fatigue. Also, the use of RO facilitates the bile flow and reduces the liver enzymes; therefore, it is used as a hepatoprotective agent and choleric in traditional medicine (Tai *et al.*, 2012, Rašković *et al.*, 2014, Al-Sereiti *et al.*, 1999). Moreover, several studies have been reported anti-diabetic (Soliman, 2013), anti-inflammatory (Mengoni *et al.*, 2011), antioxidant (Selmi *et al.*, 2017), renal protective (Azab *et al.*, 2014) and hepatoprotective (Sotelo-Felix *et al.*, 2002, Amin and Hamza, 2005) properties of this plant. According to available reports, the most antioxidant compounds of RO are carnosic acid and carnosol (Gutiérrez *et al.*, 2010). Bile duct ligation (BDL) is an animal model of chronic liver injury (Chen *et al.*, 2009, Kabirifar *et al.*, 2017a, Heidari *et al.*, 2017) that the morphological changes in this model animal are similar to human cholestasis/cirrhosis (Heidari *et al.*, 2017). Considering the presence of antioxidant compounds such as carnosic acid and carnosol in RO plant and the role of oxidants in the pathogenesis of liver cholestasis induced by BDL, in the current study, the hydroalcoholic extract of this plant was used to investigate its potential protective effects against BDL induced liver cholestasis.

MATERIALS AND METHODS

Preparations of *Rosmarinus officinalis* hydroalcoholic extract: Plant leaves were collected from forests around Yasuj in Iran, and were then cleaned and dried in the room with direct air for several days. Afterwards, they were crushed and powdered. In the next step, 100 g of the powder was added to 1000 ml of the solvent (70% ethanol). The resulting mixture was placed at 37 °C for 48 h. The solution was then filtered with Whatman filter paper Grade 1. The resulting mixture was condensed by rotary under a vacuum condition, and the extract was then dried at 50 °C of an incubator and finally kept at -20 °C.

Animals and experimental protocols: In the present study, 34 male Wistar rats weighing from 200 g to 250 g were used and adapted for 1 week before the experiments. The rats were kept in the animal centers of

Yasuj University of Medical Sciences in a 12-h light/dark cycle while having free access to rat feed diet and drinking water. All experimental procedures were approved by the current ethical considerations of local ethical committee (Grant number code: IR.YUMS.REC.1396.129) and animals were used according to "Principles of Laboratory Animal Care" (NIH Publication No. 86-23). Animals were divided into four groups as follows: sham control (SC, N=7), BDL alone (N=9), BDL+500 mg/kg BW hydroalcoholic extract of RO (N=9), and BDL+50 mg/kg BW silimarin. Rats were anesthetized intraperitoneally with ketamine HCl 50 mg/kg and xylazine HCl 10 mg/kg before surgical procedure. In brief, the general bile duct was exposed to sterile condition via a midline abdominal incision. It was then carefully double-ligated with 3-0 silk and sectioned between ligatures. Sham group rats received an identical laparotomy and handling of the common bile duct without any ligation. Treated rats received daily hydroalcoholic extract of RO 500 mg/kg/day or silimarin 50 mg/kg/day orally for 14 days after the surgery. Finally, 14 days after the BDL operation, the blood was collected by puncturing the heart under the deep anesthesia, and the animals were sacrificed. Liver tissue from each rat was removed and divided into two parts as follows: the first part was used for preparing a homogenized tissue, and the second part was fixed with 10% formalin for histology.

Biochemical examinations: Blood samples were collected in heparin-containing tubes and centrifuged at 3000 rpm for 10 min. Subsequently, the plasma was removed and froze at -20 °C for a biochemical analysis. Plasma total protein (TPRO), alkaline phosphatase (ALP), alanine aminotransferase (ALT), and aspartate aminotransferase (AST) as indicators of a liver injury were measured by standard diagnostic kits (Pars Azmoon, Iran).

Oxidative stress markers: Liver tissue was homogenized (10%, w/v) using a homogenizer (IKA Werke Ultra-Turrax T25 basic homogenizer, Germany) in PBS (10 mmol L⁻¹, pH 7.4) after cutting off the liver into small pieces. The homogenate was centrifuged at 10000×g for 10 min at 4 °C. The amounts of ferric reducing antioxidant power (FRAP), protein carbonyl (PCO), and total thiol (tSH) content were spectrophotometrically determined in the supernatant of liver tissues.

Determination of PCO content: Homogenate and plasma samples were incubated at the room temperature with 2,4-dinitrophenylhydrazine (10 mmol L⁻¹) in HCl 2 mol L⁻¹ for 1 h. 50% trichloroacetic acid was then added to the microtubes and washed by a 1:1 ethanol/ethyl acetate solution. Afterwards, the guanidinium hydrochloride solution (6 mol L⁻¹) was added and

samples were incubated at 37 °C for 15 min. Finally, the absorbance of supernatant was read at 370 nm; and the PCO content was calculated using a molar absorption coefficient of $2.2 \times 10^4 \text{ mol L}^{-1} \cdot \text{cm}^{-1}$ (Sadeghi *et al.*, 2019).

Determination of tSH: Determination of tSH group is based on the reaction of the 5,5'-dithiobis-(2-nitrobenzoic acid) or DTNB with thiol groups to obtain the mixed 2-nitro-5-thiobenzoic acid (TNB) and disulfide. In brief, tSH groups were measured by adding 25 μL of sample, 10 μL of DTNB, 150 μL of Tris-EDTA, and 790 μL of absolute methanol. The test tube was mixed gently using the pipette and kept at the room temperature for 15 min. The absorbance of supernatant was determined at 412 nm, and tSH groups were calculated using a molar absorption of $13,600 \text{ mol L}^{-1} \cdot \text{cm}^{-1}$ (Azarmehr *et al.*, 2019).

Determination of nitric oxide (NO) metabolite: Nitrite level was measured in plasma as an index of NO formation according to the Griess reaction (Sadeghi *et al.*, 2019).

Determination of the FRAP: FRAP was determined according to Benzie and Strain method (Benzie and Strain, 1996). In brief, 33 μL of sample was added to 1000 μL of FRAP reagent [300 mmol L^{-1} acetate buffer with the pH of 3.6, 10 mmol L^{-1} TPTZ (2,4,6-Tri(2-pyridyl)-s-triazine) in 40 mmol HCL , and 20 mmol L^{-1} $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ in the ratio of 10:1:1] and incubated at 37 °C for 10 min. The absorbance of the resulting blue color was measured against the reagent blank (1000 μL of FRAP reagent + 33 μL of distilled water) at 593 nm.

Determination of Malondialdehyde (MDA): Malondialdehyde was determined in plasma samples as thiobarbituric acid-reactive substances (TBA-RS) according to Ohkawa *et al.* method (Ohkawa *et al.*, 1979).

Determination of antioxidant enzyme activities: The activities of antioxidant enzymes GPX, SOD, and CAT in a homogenized tissue were measured using ELISA kits

(ZellBio GmbH, Ulm, Germany) based on the kit instructions.

Histological evaluation: Histopathologic evaluation was done by taking a liver tissue and fixing in 10% formalin buffer. In the next step, the samples were dehydrated using alcohol, and embedded in paraffin. Afterwards, 5 micron sections were prepared and stained with hematoxylin & eosin staining.

Statistical analysis: The statistical analysis was conducted using SPSS software (V.17). The Kolmogorov- Smirnov test was done for the assessment of normality. The quantitative variables including biochemical and oxidative stress markers were evaluated by one-way ANOVA with Tukey's and LSD post hoc tests. A P -value ≤ 0.05 was considered as the statistical significance.

RESULTS AND DISCUSSION

Bile duct ligation causes almost similar changes to those in humans; hence, this model was commonly used to induce cholestasis and biliary cirrhosis in animals (Colares *et al.*, 2016). In the present study, BDL model was used to investigate hepatoprotective and antioxidant effects of a hydroalcoholic extract of RO on liver cholestasis. In BDL rats, liver changes were improved during the first 4 days and increased markedly during 7 to 14 days after the surgery (Ohta *et al.*, 2003).

In the present study, amounts of AST, ALT, and ALP enzymes in the BDL group indicated a significant increase compared to the SC group (P -value ≤ 0.05 , **Table 1**). The ALP enzyme is present in the hepatocyte plasma membrane, when bile acids accumulate in the liver; it acts as a detergent, thereby releasing enzymes from the plasma membrane of hepatocytes. Bile acids also increase the ALP synthesis. Aminotransferase enzymes such as ALT and AST are found within the hepatocyte cytoplasm; hence, amounts of these enzymes do not increase to the same amount in the liver cholestatic disease.

Table 1. Effect of hydroalcoholic extract of *Rosmarinus officinalis* on plasma biochemical parameters in BDL-induced rats.

Groups	ALT (U/L)	AST (U/L)	ALP (U/L)	TPRO mg/dl
SC	36.40 \pm 6.67	87.96 \pm 10.86	447.0 \pm 55.65	7.46 \pm 0.22
BDL alone	87.26 \pm 17.91 ^a	219.21 \pm 16.98 ^a	1266.38 \pm 188.20 ^a	7.64 \pm 0.13
BDL+ RO	44.23 \pm 12.40 ^b	136.31 \pm 24.14 ^b	751.12 \pm 77.98 ^b	7.66 \pm 0.22
BDL + S	37.33 \pm 10.74 ^b	167.56 \pm 23.10	675.15 \pm 158.76 ^b	6.59 \pm 0.15

ALT: alanine aminotransferase; AST: aspartate aminotransferase; ALP: alkaline phosphatase; TPRO: total protein. Each value represents the mean \pm SEM. SC: sham control; BDL: Bile duct ligation; RO: 500 mg hydroalcoholic extract of *Rosmarinus officinalis*; S: 50 mg silimarin. ^aSignificantly different from that of the SC group ($p \leq 0.05$). ^bSignificantly different from that of the BDL alone group ($p \leq 0.05$).

In the histopathological evaluation, the SC group showed normal cellular structures with obvious liver cells, while nuclei were clearly observed. In the BDL group, tissue fibrosis, severe biliary duct hyperplasia and inflammatory infiltration of white blood cells were observed in comparison with the SC group. In

the BDL group receiving the hydroalcoholic extract of RO, moderate bile duct hyperplasia was seen, but no tissue fibrosis was observed (**Figure 1**). The liver histology analysis confirmed biochemical results and indicated that the cholestasis occurred after 14 days of BDL.

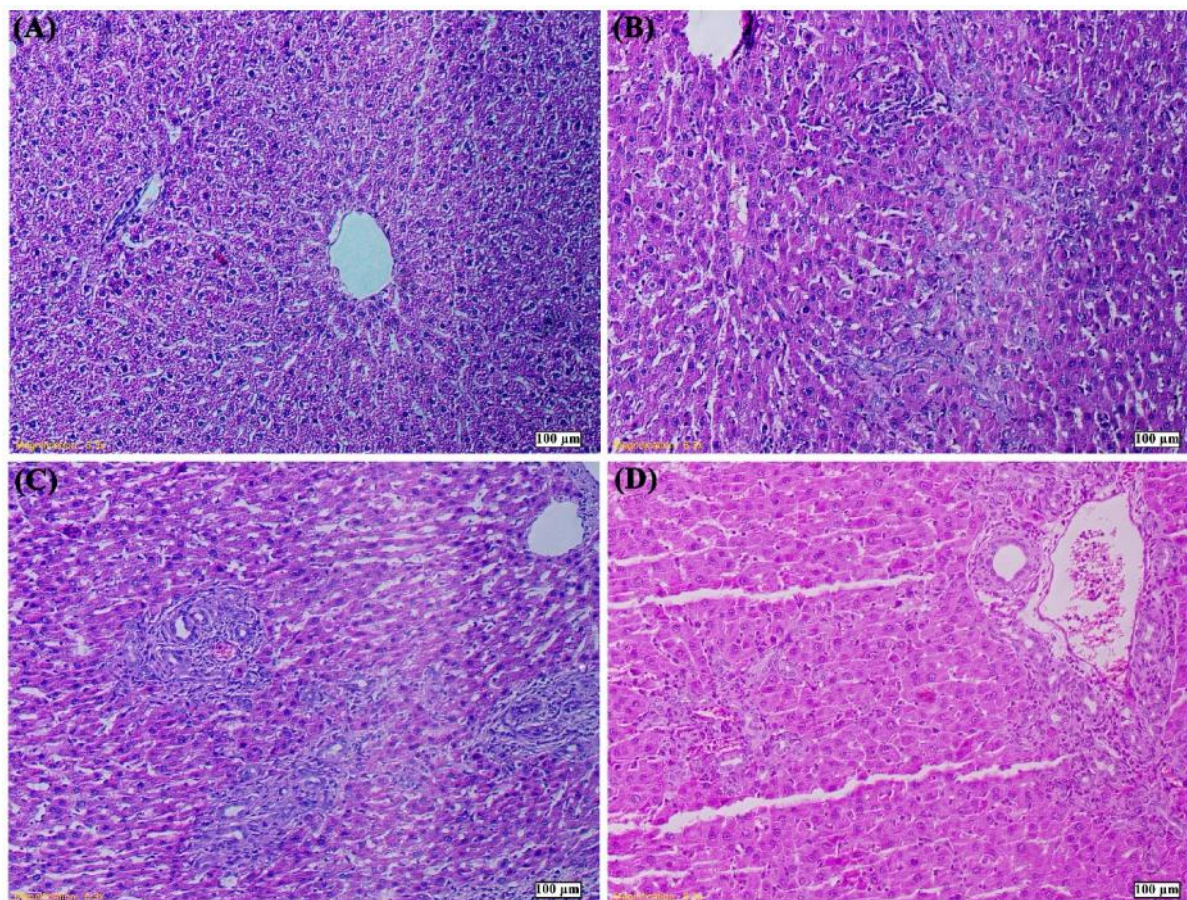


Figure 1. Photomicrograph of rat liver stained with hematoxylin and eosin (10 ×). (A) Sham control rat. (B) Bile duct ligated rat. (C) BDL rat treated with 500 mg/kg hydroalcoholic extract of *Rosmarinus officinalis*. (D) BDL rat treated with 50 mg/kg silimarin.

Rašković *et al.* (2014) indicated that the RO oil at two doses of 5 and 10 mg/day significantly decreased the levels of ALT and AST in carbon tetrachloride induced liver injury (Rašković *et al.*, 2014). Furthermore, a study by Galisteo *et al.* (2000) on the hepatic damage induced by thioacetamide and a research by Lucarini *et al.* (2014) on acetaminophen induced liver injury indicated that RO ethanolic and hydroalcoholic extracts significantly decreased ALT, AST, and ALP activities (Galisteo *et al.*, 2000, Lucarini *et al.*, 2014). In agreement with these studies, our finding showed that in the BDL group receiving 500 mg/kg RO, the values of AST, ALT, and ALP activities enzymes were significantly lower than those of BDL group (P -value ≤ 0.05 , Table 1).

It should be noted that the AST and ALT enzymes are predominantly present in cells and an increase in plasma is due to the damage to hepatocytes, resulting in the release of these enzymes into the extracellular space. In the present study, amounts of these enzymes in the BDL group receiving RO extract were markedly lower than the BDL group, which RO extract probably prevents the activation of kupffer cells by reducing the inflammatory and fibrogenic intermediates, thereby significantly inhibits the release of these enzymes (Selmi *et al.*, 2017).

Due to the imbalance between pro-oxidants and antioxidant system in cells, the oxidative stress occurs and causes damage to biomolecules such as lipids and proteins. Our findings showed that the level of liver tSH

and FRAP significantly decreased in the BDL group compared with the SC group, while liver PCO content did not show significant changes (Table 2). In addition, the levels of plasma FRAP, tSH, PCO and NO metabolite significantly increased in the BDL group compared with

the SC group ($P \leq 0.05$, Table 3). However, the treatment of hydroalcoholic extract of RO at dose of 500 mg/kg and silimarin at dose of 50 mg/kg had no significant effect on these parameters.

Table 2. Effect of hydroalcoholic extract of *Rosmarinus officinalis* on liver oxidative stress markers in BDL-induced rats.

Groups	tSH ($\mu\text{mol/g tissue}$)	PCO ($\mu\text{mol/g tissue}$)	FRAP ($\mu\text{mol/g tissue}$)
SC	7.64 \pm 0.57	2.87 \pm 0.07	1490.00 \pm 54.64
BDL alone	4.22 \pm 0.66 ^a	2.81 \pm 0.03	1103.16 \pm 91.51 ^a
BDL+ RO	4.60 \pm 0.54 ^a	2.87 \pm 0.08	1116.50 \pm 51.53 ^a
BDL + S	2.97 \pm 0.38 ^a	2.74 \pm 0.05	1055.87 \pm 84.34 ^a

tSH: total thiol group; PCO: protein carbonyl; FRAP: ferric reducing antioxidant power. Each value represents the mean \pm SEM. SC: sham control; BDL: Bile duct ligation; RO: 500 mg hydroalcoholic extract of *Rosmarinus officinalis*; S: 50 mg silimarin. ^aSignificantly different from that of the SC group ($p \leq 0.05$).

Table 3. Effect of hydroalcoholic extract of *Rosmarinus officinalis* on plasma oxidative stress markers in BDL-induced rats.

Groups	FRAP ($\mu\text{mol/L}$)	PCO ($\mu\text{mol/L}$)	MDA (nmol/L)	NO metabolite ($\mu\text{mol/L}$)	tSH ($\mu\text{mol/L}$)
SC	879.00 \pm 50.94	3.77 \pm 0.13	70.51 \pm 21.37	9.08 \pm 1.25	4.06 \pm 0.67
BDL alone	1451.08 \pm 128.59 ^a	4.49 \pm 0.06 ^a	97.48 \pm 18.34	17.37 \pm 1.20 ^a	7.25 \pm 0.96 ^a
BDL+ RO	1502.33 \pm 69.52 ^a	4.53 \pm 0.10 ^a	119.12 \pm 15.08	19.68 \pm 1.39 ^a	7.63 \pm 0.66 ^a
BDL+ S	1541.50 \pm 100.54 ^a	4.43 \pm 0.21 ^a	129.80 \pm 4.42	19.26 \pm 2.04 ^a	7.14 \pm 0.59

FRAP: ferric reducing antioxidant power; PCO: protein carbonyl; MDA: malondialdehyde; NO metabolite: nitric oxide metabolite; tSH: total thiol group. Each value represents the mean \pm SEM. SC: sham control; BDL: Bile duct ligation; RO: 500 mg hydroalcoholic extract of *Rosmarinus officinalis*; S: 50 mg silimarin. ^aSignificantly different from that of the SC group ($p \leq 0.05$).

MDA is a tissue injury index produced in BDL-induced rats due to the toxicity caused by ROS (Catalá, 2012). In a study by Alaca *et al.* (2017), MDA levels in the BDL group were higher than the control group (Alaca *et al.*, 2017). Zhao *et al.* (2017) found that the levels of MDA augmented after surgery in BDL rats (Zhao *et al.*, 2018). Abu Taher *et al.* (2016) studied liver injury induced by carbon tetrachloride (Abu Taher *et al.*, 2016), and Selmi *et al.* (2016) studied alloxan-induced diabetes (Selmi *et al.*, 2017). They found that the RO extract significantly decreased MDA. In the present study, MDA slightly increased in the plasma of rats in the BDL group; however, the infusion of RO extract into BDL rats did not have any significant effect on plasma MDA levels.

Protein carbonyl has been used as a marker for reactive oxygen-mediated alterations in proteins (Bayr, 2005). In a study by Terzioglu *et al.* (2016), the mean level of PCO in the BDL group was significantly higher than the control group (Terzioglu *et al.*, 2017). In consistent with these studies, the results of the present study indicated that the PCO level in the BDL group significantly increased compared with the SC group, while the administration of RO hydroalcoholic extract (500 mg/kg) did not show any significant effect on this factor.

Total thiol (tSH) is a sensitive indicator of oxidative stress that plays a vital role in the defense system against the ROS (Terzioglu *et al.*, 2017). In consistent with the present study, Kabirifar *et al.* (2017) found that the tSH level of liver tissue in BDL rats significantly decreased compared with SC group (Kabirifar *et al.*, 2017b). Selmi *et al.* (2017) studied alloxan-induced diabetes and found that the RO extract significantly increased tSH in the liver tissue (Selmi *et al.*, 2017). Our findings revealed that hepatic tSH levels were significantly higher in BDL rats as compared with the SC group, while the treatment of RO had no significant effect on it.

Nitric oxide, a molecule with a half-life of only few seconds, is a highly reactive mediator produced in the liver by varied cells such as endothelial, macrophage, hepatocyte, and Kupffer. Studies indicated that this compound was a primary mediator of liver damage (Aksu *et al.*, 2010). Ebrahimkhani *et al.* (2005) indicated a significant increase in NO (nitrite and nitrate) metabolites in BDL-induced cholestasis rats compared with the control group (Ebrahimkhani *et al.*, 2005). The present study indicated that the amount of NO metabolite in the BDL group was significantly higher than that of SC group, and it was consistent with the previous study

(Doustimotlagh *et al.*, 2018). However, the RO extract did not effect on this index. In contrary with the results of the present study, Gutiérrez *et al.* (2009); (Gutiérrez *et al.*, 2010) and Abu Taher *et al.* (2016) indicated that the RO extract significantly decreased NO levels in carbon tetrachloride-induced rats (Abu Taher *et al.*, 2016). This difference may be due to the different applied animal models, the research duration, and the type of applied extract.

For a comprehensive overview of what occurs in plasma, the total antioxidant status was measured using a FRAP test. In addition, the FRAP level in the liver tissue in the BDL group was significantly lower than the SC group that may be due to the higher production of oxidative agents in the liver tissue. However, the BDL group receiving RO extract did not show any significant changes in FRAP levels in the liver and plasma compared with the alone BDL group.

Table 4. Effect of hydroalcoholic extract of *Rosmarinus officinalis* on the antioxidant enzymes activity of GPX, SOD and CAT in BDL-induced rats.

Groups	CAT (U/ml)	GPX (U/ml)	SOD (U/ml)
SC	25.80 ± 0.84	439.45 ± 55.89	31.40 ± 11.15
BDL alone	29.54 ± 0.51 ^a	152.34 ± 71.60	28.80 ± 0.42
BDL+ RO	29.41 ± 0.54 ^a	278.32 ± 91.70	28.86 ± 3.60
BDL + S	28.12 ± 0.40	439.45 ± 131.24	26.94 ± 7.50

CAT: Catalase; GPX: Glutathione Peroxidase; SOD: Superoxide Dismutase. Each value represents the mean ± SEM. SC: sham control; BDL: Bile duct ligation; RO: 500 mg hydroalcoholic extract of *Rosmarinus officinalis*; S: 50 mg silimarin. ^aSignificantly different from that of the SC group (p≤0.05).

Previous studies indicated that the activities of CAT, SOD, and GPX enzymes decreased in 7, 10, and 45 days after the creation of BDL (Orellana *et al.*, 2000, Ale-Ebrahim *et al.*, 2015, Mansourian *et al.*, 2018). Kim *et al.* (2013) and Parola *et al.* (2001) reported that CAT and SOD activities rapidly increased following the BDL operation (Kim *et al.*, 2013, Parola and Robino, 2001). In accordance with Kim *et al.*' study, the present study indicated that the CAT activity in the BDL group significantly increased compared with the SC group, while the GPX activity insignificantly decreased. Compared with the alone BDL group, GPX activity increased slightly in the RO recipient's BDL group. In consistent with results of the present study, Ozcelik *et al.* (2014) found that the RO ethanolic extract did not have any effect on the SOD activity (Ozcelik *et al.*, 2014).

Studies have been conducted on the effects of RO on various liver injury models. Previous studies found that the RO methanol extract protected against the acute liver injury caused by carbon tetrachloride (Sotelo-Felix *et al.*, 2002), and the aqueous RO extract inhibited the acute liver damage induced by azathioprine (Amin and Hamza, 2005). Furthermore, the RO methanol extract could inhibit carbon tetrachloride-induced liver cirrhosis in experimental models (Gutiérrez *et al.*, 2010). Despite

CAT is a homo-tetramer enzyme that converts hydrogen peroxide into water and oxygen. The SOD enzyme is another key antioxidant defense enzyme that accelerates the conversion of radical anion superoxide to hydrogen peroxide and molecular oxygen (Brioukhanov *et al.*, 2006). GPX is an intracellular antioxidant enzyme that catalyzes hydrogen peroxide to H₂O for removing its harmful effects (Prasad *et al.*, 2015). Our findings showed that CAT activity significantly increased in the BDL group compared with the SC group (P≤0.05); however, the administration of the hydroalcoholic extract of RO to the BDL group had no significant effect on this enzyme (Table 4). GPX activity in the BDL group insignificantly reduced compared with the SC group. Nonetheless, SOD activity in the BDL group did not significant change compared with the other groups.

various studies on the effects of RO protection on liver damage, there is no study on the impact of this plant extract on BDL-induced liver cholestasis.

Conclusion: In summary, results of the present study indicated that the hydroalcoholic extract of RO plant through its effect on conventional biochemical tests such as ALT, AST and ALP, as well as the improvement of histological parameters reduced BDL-induced liver damage in rats. Despite a significant increase in the levels of oxidative stress markers in the BDL group, the hydroalcoholic extract of RO at a dose of 500 mg/kg/BW had no significant effect on these parameters.

Conflict of interest: No conflict of interest

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