



Basic nutritional investigation

Hepatoprotective effects of curcumin in rats after bile duct ligation via downregulation of Rac1 and NOX1



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ARTICLE INFO

Article history:

Received 23 March 2016

Accepted 5 June 2016

Keywords:

Curcumin

Liver damage

Rac1

NOX1

ABSTRACT

Objectives: New evidence has proven the hepatoprotective activity of curcumin; however, its underlying mechanisms remain to be elucidated. The aim of this study was to investigate the protective effect of curcumin on hepatic damage by measuring the antioxidant capacity and expression level of Rho-related C3 botulinum toxin substrate (Rac1), Rac1-Guanosine triphosphate (Rac1-GTP), and NADPH oxidase 1 (NOX1) in biliary duct-ligated (BDL)-fibrotic rat model.

Methods: Wistar rats weighing 200 to 250 g were divided into four groups (n = 8 for each): sham group, sham+Cur group (received curcumin 100 mg/kg daily), BDL+Cur group, and BDL group. The mRNA and protein expression levels of Rac1, Rac1-GTP, and NOX1 were measured by real-time polymerase chain reaction and Western blotting, respectively.

Results: Curcumin treatment of BDL rats reduced liver injury, as verified by improvement of hepatic cell histologic alterations, and by reduction of hepatic enzymes. Moreover, the increase in the expression of Rac1, Rac1-GTP, and NOX1 observed in BDL rats was precluded and reversed back toward normalcy by curcumin treatment ($P < 0.05$). We also observed an escalation of protein thiol groups, increased enzyme activity of serum antioxidant markers (e.g., superoxide dismutase) and a decrease of carbonylation in curcumin-treated BDL rats compared with BDL rats ($P < 0.05$).

Conclusions: Curcumin attenuated liver damage through the downregulation of Rac1, Rac1-GTP, and NOX1 as well as reduced oxidative stress in the serum and liver tissue of BDL rats.

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Introduction

Bile duct ligation (BDL) is an experimental model of induced cholestasis, which develops hepatocellular damage and fibrogenesis, and ultimately results in liver fibrosis [1]. During liver fibrogenesis, quiescent hepatic stellate cells (HSCs) become active and undergo a transformation that is associated with enhanced cell proliferation and overproduction of extracellular matrix (ECM) [2,3]. Oxidative stress reflects an imbalance between the systemic manifestation of reactive oxygen species (ROS) and a biological system's ability to detoxify the reactive

intermediates or to repair the resulting damage. Oxidative stress increases the HSC transformation to activated HSCs named myofibroblasts (MFs), and induces fibrosis [4]. Recent studies revealed that oxidative stress resulting from the metabolism of BDL plays a key role in the progress of hepatic fibrosis [5]. The imbalance can arise from the lack of antioxidant capacity or from an excess of ROS from other factors.

Among the different molecules involved in ROS production during liver damage, a critical role is played via the NADPH oxidase (NOX) complex [6–8]. NOX catalyzes the NADPH-dependent reduction of molecular oxygen to generate superoxide, which can transform to form secondary metabolites including hydrogen peroxide and hypochlorous acid (HOCl). Among the NOX family, NOX1, NOX2, and NOX4 are expressed on HSCs and may contribute to liver fibrosis [9]. NOX1 knockout HSCs produce less ROS than NOX2 knockout HSCs; hence, NOX1 seems to play more crucial role in ROS generation in HSCs than NOX2 [4,10].

The funding of this project was supported by Shahid Sadoughi University of Medical Sciences, Yazd, Iran. This article was extracted from Z-a-s G's Master's thesis.

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Rho-related C3 botulinum toxin substrate (Rac1) is one of the central mediators of wound healing by promoting fibrosis. In a hepatic fibrosis model, Rac1 promoted NOX activation, ROS generation, and subsequent liver fibrosis [11,12]. Rac1 and Rho GTPase have been implicated in various diseases such as atherosclerosis, hypertension, cancers, and fibrosis formation [4, 13], and have several downstream effectors such NOX or cell surface receptor-mediated signaling pathways that induce inflammatory responses [11,12]. Numerous stimuli have been identified with the ability to induce activation of Rac1 and formation of Rac1-GTP (biologically activate form of Rac1), including cytokines, growth factors, mechanical stress, and oxidative stress. Rac1-GTP translocation to the membrane-bound cytochrome complex induces the production of enzymatically active NOX1 and NOX2 [4,6].

Curcumin (diferuloylmethane) is a secondary metabolite of turmeric, derived from *Curcuma longa* [14,15]. Extensive research on curcumin over decades provided greater understanding into its medicinal and health benefits. Curcumin has several pharmacologic effects and can be used as an effective medicine for various disorders such as asthma and hepatic disease [16]. There are many reports of its antioxidant, antiinflammatory, hepatoprotective, cardioprotective, and antifibrosis activities. The molecular mechanisms underlying the targets of curcumin are varied and involve combinations of multiple signaling pathways [4,17,18]. However, the molecular mechanism of the curcumin effect on liver fibrosis remains largely unidentified.

According to the evidence that Rac1 and NOX1 expressions are associated with oxidative stress and hepatic fibrogenesis, in the present study we aimed to evaluate the effects of curcumin on Rac1, Rac1-GTP, and NOX1 expression in a BDL fibrotic rat model. As the markers of oxidative stress, we also quantified protein carbonylation and total reduced thiols (glutathiones) and assessed enzymatic activities of superoxide dismutase (SOD) and catalase (CAT).

Materials and methods

Animals and experimental procedures

Adult male Wistar rats (200–250 g, Pasteur Institute, Tehran, Iran) were used in this research. Rats were housed in an air-conditioned room at 25°C with a 12-h dark/light cycle and had free access to rat food diet and drinking water. All of the study's protocols fulfilled the current ethical considerations of local ethical committee of animal use.

Thirty-two rats were randomly detached in two groups: a sham group and a BDL group. Each group was separated into two subgroups for treatment with either daily curcumin 100 mg/kg (Sigma Chemicals Co., St. Louis, MO, Purity [high-performance liquid chromatography] >80%, USA) suspended in 5% carboxymethyl cellulose (CMC) [19,20] or the same volume/weight of the 5% CMC vehicle by oral gavage once a day beginning the day after surgery for 28 d [20]. BDL was accomplished as described previously [21]. Concisely, under general anesthesia by ketamine (90 mg/kg) and xylazine (10 mg/kg) intraperitoneally, the common bile duct was exposed by a midline abdominal incision under sterile conditions. It was then ligated in two places with a silk thread and sectioned between the ligatures [22] and Cefazolin antibiotic was used to prevent infection.

At the end of the 4-wk period, blood samples were collected by puncturing the heart under deep anesthesia and then centrifuged at 3000g for 15 min. The serum was isolated for further experiments. Liver tissues were divided into three parts, one part was frozen in liquid nitrogen for RNA extraction, the second part was kept at –70°C to make homogenized tissue for assessment of oxidative stress parameters, Rac1, Rac1-GTP, NOX1 Western blot analyses, and the last one was fixed with 10% neutral formalin for histology.

Histopathologic evaluation

The liver specimens were fixed in 10% neutral formalin individually, dehydrated in alcohol and embedded in paraffin; sections were stained with hematoxylin and eosin (H&E). A pathologist examined lobular and portal inflammation, focal hepatocyte necrosis, ductular proliferation, portal and septal

fibrosis. Rats in the BDL group with no significant histopathologic changes were excluded from the study.

Blood chemistry

The collected serum was examined for alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) as indicators of the hepatic function using standard animal diagnostic kits (Pars Azmon Diagnostic Co., Iran) and a Roche (Indianapolis, IN, USA) BT3000 Auto Analyzer.

Hepatic hydroxyproline content

An automated procedure for quantitative assay of hydroxyproline in tissue is based on the oxidation of hydroxyproline by chloramine T in aqueous solution. The oxidation product reacts with Ehrlich's reagent, and the chromogen obtained is registered in a recorder connected to the colorimeter. The amount of hydroxyproline is expressed as µg/mg tissue [23].

Determination of thiol groups in liver tissue

Ellman's reagent 5, 5'-dithiobis (2-nitrobenzoic acid) (DTNB) was first introduced for the assessment of free thiol groups. The procedure is dependent on the reaction of the thiol with DTNB to give the mixed disulfide and 2-nitro-5-thiobenzoic acid (TNB), which is quantified via the absorbance of the anion (TNB²⁻) at 412 nm [24]. Free thiol groups were measured by adding 50 µL Tris, 25 µL DTNB, and 420 µL water (495 µL initial volume take blank) and +5 µL of sample (final volume of 500 µL). The solution was mixed gently using pipette. The cuvette was placed into ultraviolet-Vis spectrophotometer and reading was taken at 412 nm. Absorbance was taken for each homogenized tissue sample, and the results were expressed in nmol/mg protein.

Determination of protein carbonylation in liver tissue

Homogenized tissue samples were treated with in 2.5 N HCl, 10 Mm 2,4 dinitrophenylhydrazine for 1 h in the dark at room temperature. After treatment with 20% trichloroacetic acid and separation via centrifugation, the precipitate was washed three times with a mixture of absolute ethylic alcohol and ethylic acetate 1:1(v/v). Subsequently, protein precipitate was dissolved in 6 M guanidine hydrochloride. Protein concentration was quantified in these samples through measuring the absorbance at 355 nm, and the results were expressed in nmol/mg protein.

Catalase activity in liver tissue

CAT activity was assayed by previously described methods [25,26] in which the vanishing of peroxide is followed spectrophotometrically at 240 nm. The incubation mixture contained 0.05 M potassium phosphate, pH 7.0, 0.020 M hydrogen peroxide, and a sample (0.05 mL) of the supernatant fluid, in a final volume of 3 mL. The decrease in absorbance was recorded at 240 nm for 3 min. The rate of decrease in absorbance per minute was calculated from the initial linear portion of the curve. The value of 0.0394 cm⁻¹ M⁻¹ previously was used as the destruction coefficient of H₂O₂ [26]. One unit of CAT was defined as the amount of enzyme that decomposed 1 mol/min of H₂O₂ at 25°C and pH 7.0 under the specified conditions, and the results were expressed in IU/mg protein.

SOD activity in liver tissues

The principle of SOD activity measurement was based on the inhibition of nitroblue tetrazolium (NBT) reduction. Illumination of riboflavin in the presence of oxygen and methionine (electron donor) generates superoxide anions and this has been introduced as the basis of the assay of SOD. The reduction of NBT by superoxide radicals to blue-colored formazan was recorded at 560 nm, as previously described [27]. The reaction mixture contained 1.9 mL of phosphate buffer [pH 7.8], 16.8 × 10⁻⁵ M NBT, 1.17 × 10⁻⁶ M riboflavin, and 10⁻² M methionine, with suitably diluted homogenized tissue in a volume of 3 mL. The absorbance was recorded at 560 nm for 5 min. The rate of increase in absorbance per minute was calculated from the initial linear portion of the curve. The value of 0.00436 cm⁻¹ mol⁻¹ was used as the extinction coefficient. The values were expressed in IU/mg protein.

Western blot analysis of liver tissue Rac1, Rac1-GTP, and NOX1 levels

Liver cell protein was extracted by homogenization of tissue samples (30 mg) using phosphate saline buffer (100 mM Tris-HCl, 150 mM NaCl, 0.1% sodium dodecyl sulfate [SDS], and 1% NP-40; pH 7.4 with protease-inhibitor cocktail, 1:100; Sigma, St. Louis, MO, USA) by incubation on ice for 30 min and subsequent centrifugation at 15 000g (4°C, 30 min). Protein concentrations were determined in the supernatants by the Bradford assay. Protein (100 µg) was separated on a

12% SDS-polyacrylamide gel and transferred onto a nitrocellulose membrane. The membrane was blocked with 3% nonfat dried milk in Tris-buffered saline, pH 7.4, with 0.05% Tween-20 (TBS/T) for 2 h and probed with monoclonal rabbit anti-Rac1 primary antibody (Abcam, Cambridge, UK), polyclonal rabbit anti-NOX1 (Abcam, Cambridge, UK), monoclonal mouse anti-Rac1-GTP (Newest biosciences) and polyclonal rabbit anti- β -actin (Abcam, Cambridge, UK) as a reference at 4°C overnight. The membranes were incubated with a goat antirabbit and antimouse immunoglobulin G secondary antibody (1:4000) conjugated with horseradish peroxidase (cell signaling, Munich, Germany) for 45 min. The predicted sizes for Rac1 (21 kDa), Rac1-GTP (~22 kDa), NOX1 (71 kDa), and β -actin (42 kDa) were checked using molecular-weight markers. Specific bands were visualized by an enhanced chemiluminescence reagent (GE) on a ChemiDoc system (SynGene GBOX, 680 X) and quantified densitometrically with the program Quantity GeneTools (SynGene, V4.1).

Gene expression analysis by quantitative real-time PCR

Total cellular RNA was isolated from liver samples by using a Fast Pure RNA Kit from TakaRa, Japan according to the manufacturer's protocol. By measuring the absorbance at 260 nm, concentrations of RNA were identified and its purity was evaluated by 260/280 nm absorbance ratio (Eppendorf, Hamburg, Germany). One microgram of the total RNA was reverse transcribed to cDNA using MuLV RT enzyme (Fermentas), random hexamers, and dNTP in a total volume of 20 μ L. The cDNA samples were diluted 1/10, and aliquots were frozen at -70°C until the polymerase chain reactions (PCRs) were carried out. Quantitative real-time PCR (qPCR) was performed triplicate using SYBR-green in the Rotor Gene system (Corbett Research 2004, Australia). Normalization was achieved against β -actin and relative quantity of gene expression was analyzed based on $\Delta\Delta C_t$ method and the results were calculated as $2^{-\Delta\Delta C_t}$.

Oligonucleotide primer sequences used for qPCR were as follow: α -smooth muscle actin (α -SMA): forward 5'-GCTCCATCCTGGCTCTCTATC-3' and reverse

5'-GGGCCAGCTTCGTCATACTC-3'; collagen I: forward 5'-ATCAGCCCAAACCC AAGGAG-3' and reverse 5'-CGCAGGAAGTCAGCTGGATAG-3'; transforming growth factor (TGF)- β 1: forward 5'-AAGAAGTCACCCCGCTGTA-3' and reverse 5'-TGTGTGATGTCCTTGGTTTTGTC-3'; Rac1: forward 5'-GTAACCTGCTGCTCA TC-3' and reverse 5'-GCTTCATCAAACACTGCTTC-3'; Nox1: forward 5'-TACG AAGTGGCTGACTGGTTG-3' and reverse 5'-CTCCAAAGGAGGTTTTCTG-3'; β -actin: forward: 5'-CGTTGACATCCGTAAGACCTC-3' and reverse: 5'-AGCCACCG ATCCACACAGA-3'.

Statistical analyses

Differences between obtained values (mean \pm SEM) were carried out by one-way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparison using Graphpad Prism 5 software (GraphPad Software, San Diego, California, USA). $P < 0.05$ was considered statistically significant.

Results

Curcumin attenuated BDL-induced histopathologic changes

The histologic examination of liver by H&E staining revealed that sham-operated control rats had normal liver morphology with intact hepatocytes and portal tracts (Fig. 1A, B). Parenchymal necrosis and an abundance of newly formed bile ducts, including bridging of the portal tracts, were found in BDL rats (Fig. 1C). Curcumin supplementation prominently amended all of the histopathologic changes observed in the BDL group (Fig. 1D).

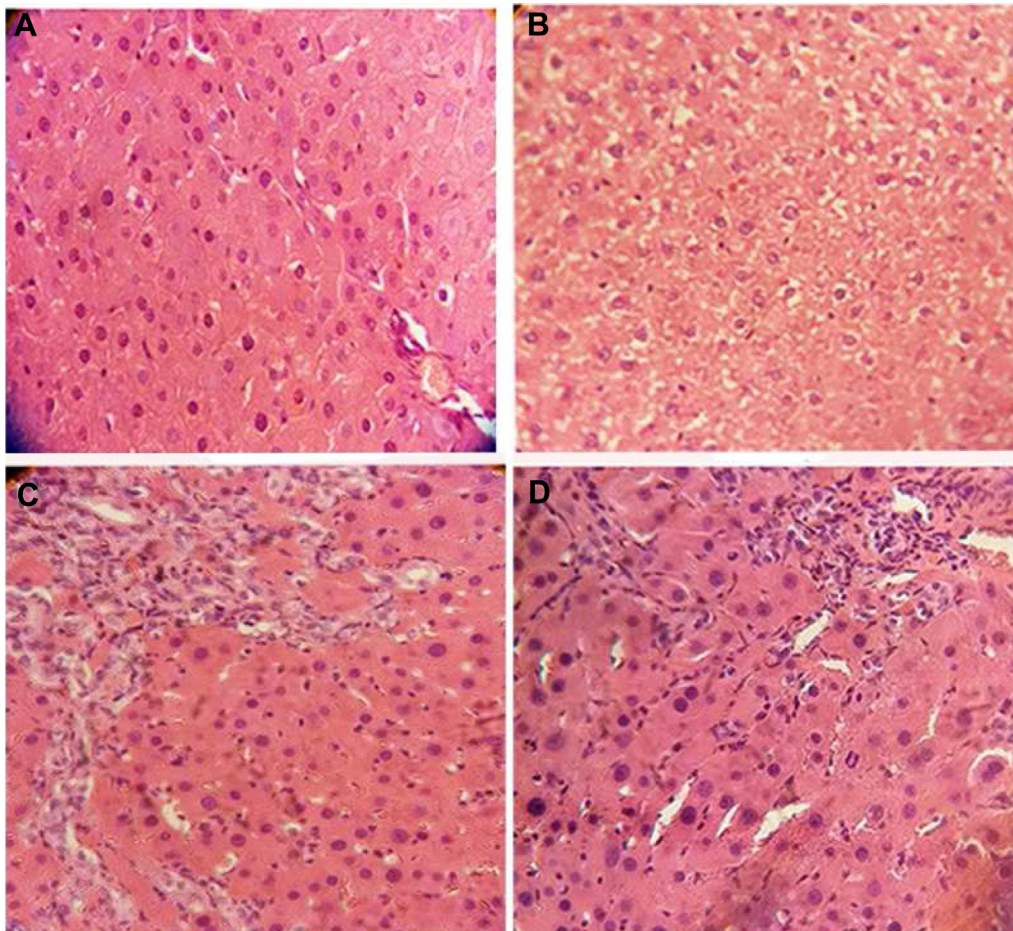


Fig. 1. Curcumin attenuated BDL-induced liver injury as shown by histologic examination. Representative photomicrographs of H&E staining are (A) sham, (B) sham+Cur, (C) BDL, and (D) BDL+Cur. BDL, bile duct ligation; Cur, curcumin; H&E, hematoxylin and eosin.

Table 1
Effect of curcumin on hepatic enzymes (AST, ALT, and ALP) in four groups of rats

	Sham	Sham+Cur	BDL	BDL+Cur
AST (U/dL)	171 ± 13.38	142 ± 6.48	488.4 ± 27.59*	310.5 ± 35.68†
ALT (U/dL)	61.5 ± 6.49	58.5 ± 3.44	150.2 ± 14.27*	138.5 ± 5.95†
ALP (U/dL)	263.5 ± 14.5	271 ± 8.11	958 ± 45.05*	559 ± 30.34†

ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BDL, bile duct ligation; Cur, curcumin

* Enzymes activity in BDL+Cur group compared with BDL group (* $P < 0.05$). The results are given as mean ± S.E.M.

† Activity of three liver enzymes (AST, ALT, ALP) in BDL group were increased compared with sham groups († $P < 0.05$).

Effect of curcumin on serum biochemistry of BDL rats

Table 1 represents the results of the curcumin on serum hepatic enzymes. Serum levels of AST, ALT, and ALP were significantly elevated in the BDL rats ($P < 0.05$). High levels of these three hepatic enzymes in BDL rats were significantly decreased after daily treatment with curcumin (100 mg/kg; $P < 0.05$).

Hydroxyproline quantification

Table 2 represents the results of the curcumin on liver tissue hydroxyproline level. The hepatic level of hydroxyproline was significantly elevated in the BDL rats ($P < 0.05$). The high level of hydroxyproline in these rats was significantly decreased after their daily treatment with curcumin (100 mg/kg; $P < 0.05$).

mRNA expression of α -SMA, collagen I, and TGF- β 1

We measured the level of mRNA expression α -SMA, collagen I, and TGF- β 1 in liver tissue of four groups (sham, sham+Cur, BDL, and BDL+Cur) by the qPCR method (Table 2). We observed that α -SMA, collagen I, and TGF- β 1 gene expressions were significantly increased in liver tissue of the BDL rats compared with the sham group ($P < 0.05$). However, curcumin treatment reduced gene expression of α -SMA, collagen I, and TGF β 1 in the liver tissue of the BDL+Cur group compared with the BDL group.

Curcumin attenuated BDL-induced oxidative stress

Thiol groups and carbonyl were measured as two indicators of damage to liver tissue proteins and determined the activity of CAT and SOD enzymes, two important enzymes in the antioxidant defense. In the BDL group, in comparison with sham group, the activity of antioxidant enzymes (SOD and CAT; Fig. 2C, D) as well as the concentration of thiol groups (Fig. 2A) significantly decreased, whereas the concentration of carbonyl groups

Table 2
Comparison of hydroxyproline content and the expression of α -SMA, TGF- β 1, and COL 1 in liver tissue of four groups (sham, sham+Cur, BDL, BDL+Cur)

	Sham	Sham+Cur	BDL	BDL+Cur
Hydroxyproline (μ g/mg tissue)	0.91 ± 0.1	0.84 ± 0.21*	4.24 ± 0.59	2.54 ± 0.57†
α -SMA	3.23 ± 0.09	4.34 ± 0.35	6.17 ± 0.19*	3.66 ± 0.09†
COL 1	2.3 ± 0.28	2.61 ± 0.43	4.06 ± 0.21*	2.22 ± 0.03†
TGF- β 1	3.98 ± 0.24	3.88 ± 1.04	6.44 ± 0.04*	3.76 ± 0.51†

BDL, bile duct ligation; COL, collagen; Cur, curcumin; SMA, smooth muscle actin; TGF, transforming growth factor

* P value < 0.05 compared with the BDL group. The results are given as mean ± SD.

† P value < 0.05 compared with the Sham group.

(Fig. 2B) increased intensely ($P < 0.05$). These observations confirmed the presence of oxidative stress in liver tissue of the BDL rats. In contrast, curcumin treatment of the BDL rats resulted in an increase in the level of free thiol groups, and the enzyme activity of CAT and SOD in the liver tissue (Fig. 2A, C, D), whereas the carbonyl level of proteins was reduced (Fig. 2B).

mRNA expression of Rac1, Rac1-GTP, and NOX1 was decreased in curcumin-administered group

After 28 d from BDL surgery and then treatment with curcumin, we measured the level of mRNA expression Rac1 and NOX1 in liver tissue of the four groups (sham, sham+Cur, BDL, and BDL+Cur) by qPCR method. We observed that both Rac1 and NOX1 gene expressions significantly increased in the liver tissue of the BDL rats compared with sham group. However, treatment with curcumin reduced the gene expression of Rac1 and NOX1 in the liver tissue of the BDL+Cur group compared with the BDL group, as shown in Figure 3A and B.

Protein expression of Rac1, Rac1-GTP, and NOX1 was reduced in curcumin-administered group

We measured the level of protein expression Rac1, Rac1-GTP, and NOX1 in liver tissue of the four groups by Western immunoblotting to confirm their mRNA expression profile. We observed that the relative density of Rac1, Rac1-GTP, and NOX1 significantly increased in the liver tissue of the BDL rats compared with sham group ($P < 0.05$). The expression level of Rac1, Rac1-GTP, and NOX1 proteins in liver tissue of BDL+Cur rats decreased once compared with BDL rats ($P < 0.05$), as shown in Figure 4.

Discussion

The present study investigated the hepatoprotective effect of curcumin through evaluation of serum antioxidant markers, hepatic enzymes, hepatic fibrosis markers, and gene and protein expression profiles of Rac1, Rac1-GTP and NOX1 in a BDL-fibrotic rat model. To the best of our knowledge, this is the first study to determine the hepatoprotective effect of curcumin by evaluating the expression levels of NOX1, Rac1, and Rac1-GTP in BDL rats. Our findings revealed that curcumin treatment attenuated liver injury through downregulation of either mRNA or protein expression of Rac1-GTP, Rac1, and NOX1. To assess the fibrosis process of hepatic tissue during BDL, we measured mRNA expression of three fibrotic markers, including α -SMA, collagen I, and TGF- β 1, in the liver tissue of four study groups, as well as hepatic level of hydroxyproline. Compared with the sham group, BDL rats demonstrated a significant increase in the fibrosis markers, but treatment of the BDL rats with curcumin led to a reduction in these markers.

Curcumin (diferuloylmethane) is a polyphenol derived from the yellowish pigments of turmeric plant. It has beneficial properties such as anticancer, antioxidant, antiinflammatory, hepatoprotective, and antifibrosis effects [14,15]. Nevertheless, the effect of curcumin on the prevention of liver fibrosis and its molecular mechanism has not been fully understood. BDL is a representative model of biliary cholestasis in animals that results in oxidative damage. It stimulates the HSCs to secrete more collagen fibrosis and induces the accumulation of ECM leading to liver fibrogenesis [21]. We used the BDL method to induce hepatic fibrosis and confirmed the fibrosis through assessment of the levels of serum hydroxyproline, the mRNA expression of

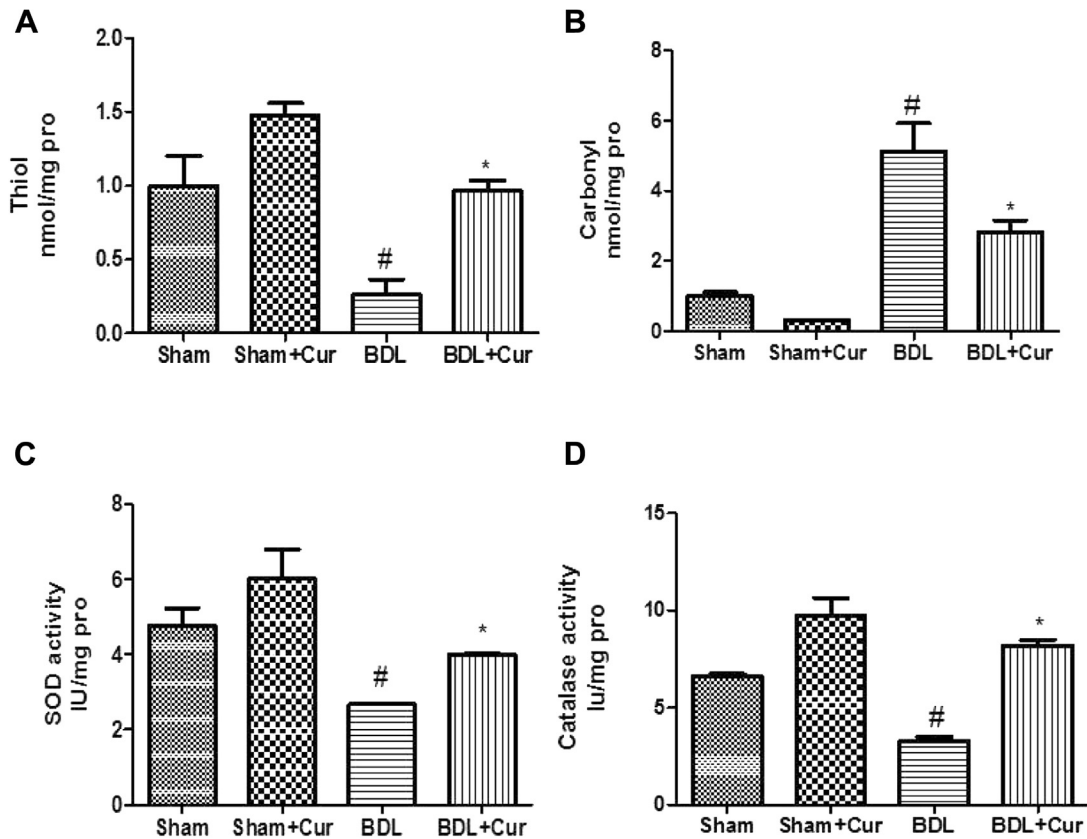


Fig. 2. Effects of curcumin on the levels of thiol group (A), carbonyl group (B), SOD activity (C) and catalase activity (D) in the liver tissue of the four groups (sham, sham+Cur, BDL, BDL+Cur). * $P < 0.05$ compared with sham group. # $P < 0.05$ compared with BDL group. BDL, bile duct ligation; Cur, curcumin; SOD, superoxide dismutase.

α -SMA, collagen I, and TGF- β 1, and by measuring hepatic enzymes including ALT, AST, and ALP, as well as H&E staining of hepatic sections of BDL rats. Based on our observation, curcumin treatment of BDL rats significantly improved fibrosis of the liver tissue and increased the activity of hepatic enzymes.

The liver is a dynamic organ with fundamental contributions in metabolism, detoxification, and elimination of endogenous and exogenous substances [28]. Huge amounts of evidence have demonstrated the implication of oxidative stress in liver damage. Oxidative stress is a disruption in the oxidant-antioxidant

balance, resulting in potential cellular damage. The imbalance can arise from an absence of antioxidant capacity caused by disturbances in production and distribution, or by an excess of ROS from other factors [29,30]. Recent studies have revealed that oxidative stress resulting from the metabolism of BDL plays a significant role in the hepatic fibrosis development [5]. Among all antioxidants that are available in the body, thiols constitute the major portion of the total body antioxidants and are responsible for the defense against ROS. Among principal plasma antioxidants available in bodily fluids, thiols are organic

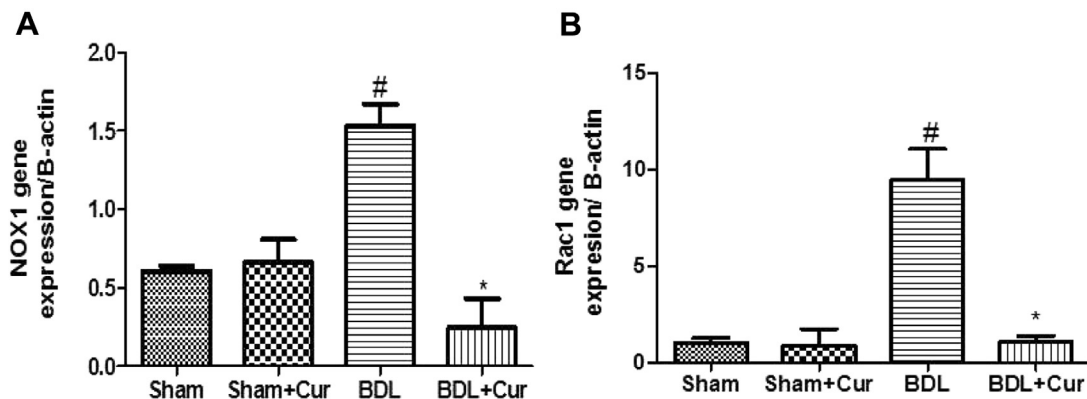


Fig. 3. Gene expression of NOX1 (A) and Rac1 (B) in four studied groups (sham, sham+Cur, BDL, BDL+Cur). * $P < 0.05$ compared with sham group. # $P < 0.05$ compared with the BDL group. BDL, bile duct ligation; Cur, curcumin.

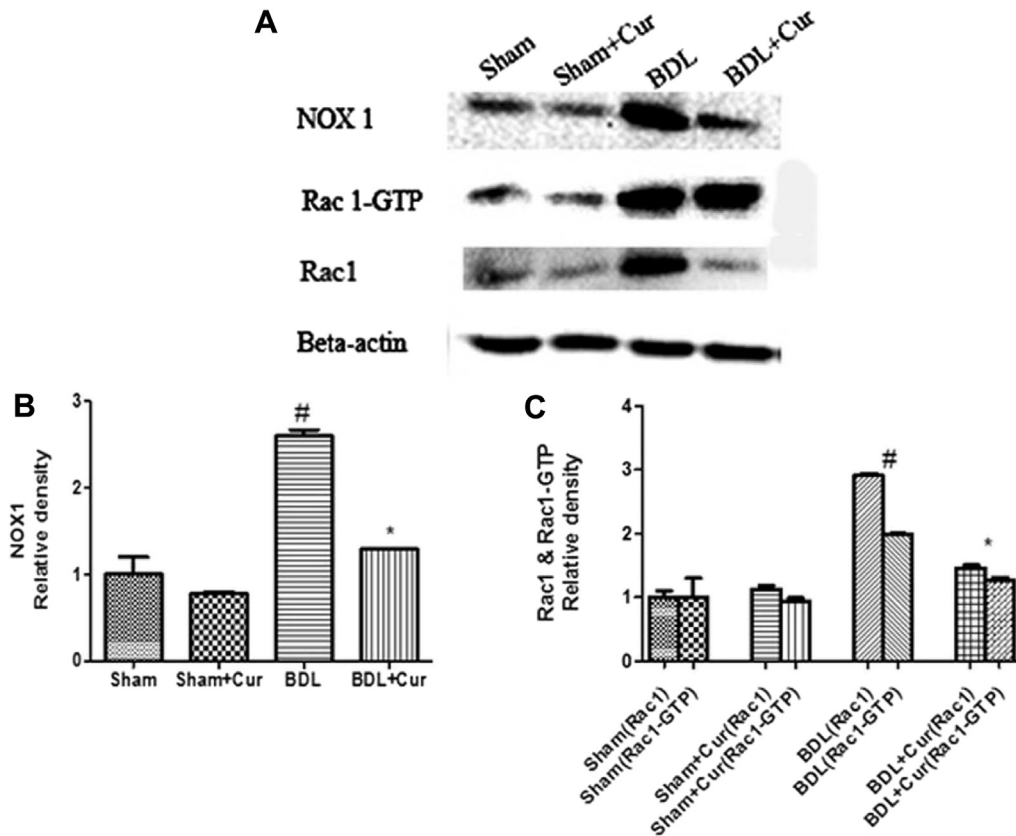


Fig. 4. Western blotting pattern of Rac1, Rac1-GTP, and NOX1 proteins expression (A); the relative density of protein expression levels of NOX1 (B) and Rac1, Rac1-GTP (C) in four studied groups (sham, sham+Cur, BDL, BDL+Cur). * $P < 0.05$ compared with sham group. # $P < 0.05$ compared with BDL group. BDL, bile duct ligation; Cur, curcumin.

compounds with essential reducing groups that fight against oxidants like ROS [31,32].

In contrast, carbonylation of proteins, which is an irreversible oxidative damage, frequently impairs proteins function. It is considered a prevalent indicator of severe oxidative damage and disease-derived protein dysfunction that can be promoted by ROS [33]. Our findings have shown that the thiol group, as the sensitive indicator of rat oxidative stress, was reduced significantly when exposed to oxidative stress caused by BDL. BDL-induced oxidative stress also led to increased production of carbonyl group on proteins' peripheral chains. In agreement with our findings, another group of researchers reported an increase in carbonyl protein levels and a decrease in thiol group in BDL rats compared with a control sham group [34]. Our experiments demonstrated that treatment of BDL rats by curcumin increases the amount of amino acid free thiol groups, whereas it reduces protein carbonylation.

Under oxidative stress conditions, the antioxidant enzymes such as SOD and CAT remove the extra ROS to maintain homeostasis. Recent studies have shown a significant reduction in the activity of CAT and SOD in BDL rats compared with control groups [35–37]. Likewise, we observed reduced levels of these enzymes in the BDL group compared with the sham group. However, the enzyme activity of CAT and SOD were increased in the BDL rats after they were treated by curcumin, resulting in a protection against hepatic fibrosis through elevation of the antioxidant capacity.

Hepatic fibrosis is a pathologic process characterized by synthesis, secretion, and accumulation of ECM derived from increased MF-HSC in the liver [2]. The required event for

accumulation of MF-HSC and induction of hepatic injury is the transportation of HSC from the passive phenotype to the MF form. Rac1 has been identified as the key mediator for accumulation of MF-HSC, associated with progression of hepatic fibrosis [38,39]. Rac1 is a subunit of cytosolic NOX1. The activated Rac1 stimulates NOX1, and the subsequent activity of NOX1 induces conversion of HSCs to MF forms, thus promoting hepatic fibrosis [39]. NOX1 is a multiprotein complex that produces ROS in response to various stimuli [40]. Activated Rac1 (Rac1-GTP) directly binds to NOX1 through tetratricopeptide repeat site and leads to the regulation of ROS producer NOX1 [41,42]. Recent observations have demonstrated that in rats with carbon tetrachloride-induced fibrosis, the Rac1 activity led to overproduction of NOX1-mediated ROS as well as increased activity of HSCs that ultimately aggravated hepatic fibrosis [39]. However, Rac1 deficiency resulted in suppression of NOX1 as well as reduction in oxidative stress [43]. Our experiments indicated that in BDL fibrotic rats the rate of NOX1 expression was significantly increased, and that this increase was in parallel to the escalation in the expression rate of Rac1 and Rac1-GTP once compared with the sham group. However, these rates were reduced upon treatment of BDL fibrotic rats with curcumin (BDL+Cur compared with the BDL group).

Conclusion

Results from the present study demonstrated that the BDL fibrotic rats expressed higher levels of Rac1, Rac1-GTP, and NOX1 but they produced a reduced level of antioxidant markers. Curcumin treatment of BDL rats led to downregulation of Rac1,

Rac1-GTP, and NOX1, and elevation of antioxidants (thiols, SOD, and CAT), hepatic enzymes (ALP, AST, ALT), and hepatic fibrosis markers (α -SMA, collagen I, TGF- β 1, and hydroxyproline). Therefore, the present study suggests that curcumin exerts its antihepatofibrotic effects via reduction of oxidative stress as well as downregulation of Rac1-GTP, Rac1, and NOX1.

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