Antioxidant, anti-inflammatory and anti-fibrotic effects of *Boswellia serrate* gum resin in CCl$_4$-induced hepatotoxicity

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Abstract. The present study aims to investigate the potential antioxidant, anti-inflammatory and anti-fibrotic effects of *Boswellia serrate* (BS) gum resin against carbon tetrachloride (CCl$_4$)-induced liver damage. Four groups consisting of eight rats each were designated: Group I, normal healthy control; group II, CCl$_4$-induced liver fibrosis; group III, CCl$_4$-induced liver fibrosis followed by BS treatment daily for two weeks; and group IV, CCl$_4$-induced liver fibrosis followed by silymarin treatment daily for two weeks. Expression of tumor necrosis factor-α (TNF-α) and nuclear factor κB (NF-κB), interleukin-6 (IL-6), transforming growth factor-β (TGF-β) and cyclooxygenase-2 (COX-2) were assessed, in addition to histopathological and fibrotic changes in liver tissues isolated from the rats. BS significantly ameliorated CCl$_4$-induced increases in serum aspartate (AST) and alanine transaminase (ALT) levels, reduced lactate dehydrogenase (LDH) activities in addition to restoring total bilirubin, triglyceride and albumin levels. BS treatment also alleviated oxidative stress and improved total antioxidant capacity in the liver, and reduced the expression of TNF-α, NF-κB, TGF-β, IL-6 and COX-2. On a histopathological level, BS treatment also exhibited antifibrotic activity. In conclusion, these findings suggest that BS contains potentially hepatoprotective effects against CCl$_4$ induced liver injury via its antioxidant, anti-inflammatory and antifibrotic characteristics.

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Introduction

Liver damage resulting from multiple factors including drug misuse, alcohol abuse and pesticides represents a major international health problem (1). To tackle this problem investigations into the potential hepatoprotective properties of food, medicinal plants and their bioactive constituents is currently of upmost importance. Indeed, inflammation and reactive oxygen species (ROS)-induced oxidative stress are major players in the pathogenesis of many chronic diseases such as liver fibrosis (2). The huge diversity of compounds derived from natural products provides a rich source of potential therapeutic agents (3). Accumulating evidence supports the beneficial antioxidant effects of food-derived phenolic compounds on the health of the liver, by protecting against chemical-induced hepatotoxicity such as ethanol-induced liver injury (4). Carbon tetrachloride (CCl$_4$)-intoxication is an animal model of oxidative stress-induced liver injury that is widely used for assessing the hepatoprotective capacity of prospective therapeutic agents (5).

One such agent, the Gum resin of *Boswellia serrata* (BS), has been used for centuries as a traditional remedy for a variety of ailments in Ayurvedic medicine. The anti-inflammatory, anti-atherogenic, and analgesic properties of BS have been recognized for centuries (6). Extracts from this gum resin have previously been demonstrated to target the humoral and adaptive immune response (7). *In vitro* studies have revealed that the boswellic acids, consisting of a group of pentacyclic triterpenoid compounds/acids, and their acetylated derivatives can inhibit the biosynthesis of pro-inflammatory mediators such as leukotrienes (8), which increase cell permeability. In particular, 3-acetyl-11-ketobeta-boswellic acid (AKBA) has been found to be a natural inhibitor of the transcription factor NF-κB, which is an important downstream mediator of cytokines during inflammation (9). These anti-inflammatory properties has been attributed to the boswellic acids (α, β and γ-boswellic acid), acetyl-β boswellic acid, 11-keto-β-boswellic acid and acetyl-11-keto-β-boswellic acid (10), which can also simultaneously reduce oxidative stress (11). This group of triterpenic acids have also been reported to exhibit anti-cancer properties, controlling cell proliferation, metastasis, invasion

and migration by targeting cell signaling components, including MAPK, NF-κB, TNF-α and ERK1/2 (12,13).

The aim of the present study was to elucidate the potential hepatoprotective effects and the mechanism of action of BS in CCl_4-induced hepatocellular damage rat models. These effects were biochemically and histologically assessed in addition to being compared with that of silymarin, a more well-known hepatoprotective compound (14).

Materials and methods

Chemicals and Plant Material. Chemicals used were all of analytical grade and were purchased from Sigma-Aldrich (Merck KGaA). BS oleo-gum resin utilized in the present study was a kind gift from Professor Dr H. P. T. Ammon, Department of Pharmacology, Institute of Pharmaceutical Sciences, University of Tuebingen, Germany (Tubingen, Germany).

Animals and experimental design. Experiments on animals were performed in accordance with the international ethical guidelines for animal care of the United States Naval Medical Research Centre, Unit no. 3, Abbaseya, Cairo, Egypt, accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International. The adopted guidelines were in agreement with 'Principles of Laboratory Animals Care' (NIH Publication no. 85-23, revised 1985). The study protocol was approved by The Research Ethics Committee of the Faculty of Pharmacy, Minya University (Minya, Egypt).

A total of 32 male Wistar rats (age, 7-8 weeks old; average body weight, 250±25 g) were obtained from the Animal House of Assiut University were utilized in the experimental procedures. All animals received professional care and were kept with a 12-h light/dark cycle at 20˚C and 45% relative humidity and had free access to water and food. Animals were randomly divided into four test groups of eight rats each, with the experimental procedures described as follows: i) Normal control group, which received two intraperitoneal (i.p.) injections of olive oil per week for six weeks; ii) CCl_4-treated group, in which liver fibrosis was induced by an i.p. injection of CCl_4 (1 ml/kg 40% CCl_4, diluted in olive oil) twice weekly for 6 weeks (15); iii) BS treatment group, in which the rats received a daily i.p. injection of BS (150 mg/kg body weight) for an additional two weeks directly after the end of the six-week CCl_4 treatment (16); and iv) Silymarin treatment group, in which the rats received a daily oral dose of silymarin (100 mg/kg body weight per oral gavage) for two weeks directly after the end of the six week CCl_4 treatment. At the end of the 8th week, rats deeply anaesthetized by i.p. injection of 100 mg/kg ketamine and 20 mg/kg xylazine were sacrificed by cervical dislocation.

Sample collection. To perform the biochemical analysis, 5 ml of blood were collected from animals that were deeply anesthetized by intraperitoneal injections of 100 mg/kg ketamine and 20 mg/kg xylazine by cardiac puncture. The blood samples were subsequently centrifuged (1,000 x g, 20 min at room temperature) with the subsequent serum isolated. Liver tissues were excised rapidly for histological investigation and RNA isolation. A buffer with the following composition was used for tissue homogenization for further protein analysis: 20 mM Tris, 100 mM NaCl, 1 mM EDTA and 0.5% Triton X-100 supplemented with protease inhibitors mix. Biuret reagent (Bio-Rad laboratories, Inc.) was used to estimate the protein content of homogenates, using bovine serum albumin as standard (Sigma-Aldrich; Merck KGaA). Homogenized tissue samples were stored at -70˚C until use.

Detection of nuclear NF-κB expression by western blot analysis. Preparation of nuclear samples was performed using Nuclear Extraction Kit (cat. no. ab113474; Abcam) according to manufacturer's protocol. A total of 50 µg of nuclear protein from each liver sample were used for western blot analysis according the protocol previously described by Abouzied *et al* (17). Briefly, protein samples were separated by 10% SDS-PAGE and then transferred to Hybond™ nylon membranes. Membranes were blocked for 2 h at room temperature in 5% non-fat milk diluted in TBS supplemented with 0.05% Tween-20 buffer followed by an overnight incubation at 4˚C with mouse monoclonal primary antibodies against rat NF-κB p65 (1:2,000, cat. no. TA336457; Origene Technologies, Inc.) and β-actin (1:2,000; cat. no. TA310155; OriGene Technologies, Inc.). Alkaline phosphatase-conjugated goat anti-mouse immunoglobulins (cat. no. AP32278AP-N; Origene Technologies, Inc.) were diluted 1:5,000 in the 10X diluted blocking buffer and used as a secondary antibody that was used for incubating the membranes for 2 h at room temperature. Visualization of protein bands was achieved by incubating the membranes with alkaline phosphatase buffer containing the substrate 1-step™ NBT/BCIP substrate solution (cat. no. 34042; Thermo Fisher Scientific, Inc.). Color reactions were stopped by rinsing the membranes with stop buffer (10 mM Tris-Cl, pH 6.0, 5 mM EDTA). Visualized bands were analyzed using ImageJ software (version 2.0.0; National Institutes of Health).

RNA extraction and reverse transcription-polymerase chain reaction (RT-PCR) for TNF-α. Total RNA was isolated from freshly dissected tissues using total RNA Kit (Hangzhou Bioer Co., Ltd.) according to manufacturer's protocol. The amount of isolated RNA was quantified by measuring absorbance at 260 nm. RT-PCR was performed using RevertAid RT reverse transcription kit (Thermo Fisher Scientific, Inc.) according to manufacturer's protocols. For the amplification of TNF-α, the following synthetic oligonucleotides were used: TNF-α forward, 5'-CAGCAGATGGGCTGTACCTT-3' and reverse, 5'-AAGTAGACCTGCCCGGACTC-3'; and GAPDH forward, 5'-AGATCCACAACGGAT-3' and reverse, 5'-TCC CTCAAGATTGTCAGCAA-3', where GAPDH served as the internal control (18). The reaction mixture, consisting of 2 µl cDNA, 2.5 U REDtaq® Readymix™ PCR Reaction Mix (Sigma-Aldrich; Merck KGaA) and 0.1 µmol/l primers (Fermentas; Thermo Fisher Scientific, Inc.) was amplified using a Biometra cycler® (Biometra GmbH). Thermocycling started with 4 min of pre-denaturation at 94˚C followed by 30 cycles of denaturation at 94˚C for 30 sec, annealing at 55˚C for 30 sec and extension at 72˚C for 1 min. Finally, the mixture was incubated at 72˚C for 4 min and cooled to 4˚C. Subsequent PCR products were separated on 1.5% (v/v) agarose gels and visualized by ethidium bromide (0.3 µg/ml final concentration)

Table I. Levels of liver biomarkers in the different test groups.

Parameter	Control (n=8)	CCl$_4$ (n=8)	CCl$_4$ + *Boswellia* extract (150 mg/kg) (n=8)	CCl$_4$ + Silymarin (100 mg/kg) (n=8)
AST (U/l)	91±4.700	240±10.800[c]	120.0±9.100[b]	105±11.300[b]
ALT (U/l)	40±2.300	108±9.700[c]	60±4.800[a]	45±3.700[b]
Bilirubin (mg/dl)	0.12±0.080	0.46±0.020[c]	0.15±0.005[b]	0.13±0.090[b]
Albumin (mg/dl)	5.1±0.410	2.9±0.170[c]	4.3±0.250[a]	4.8±0.180[b]
Triglycerides (mg/dl)	73±6.300	130±10.200[c]	81±4.700[b]	75±5.400[b]
Cholesterol (mg/dl)	83±3.22	152±5.700[c]	94 ±8.300[b]	83.00±5.150[b]

Values represent the mean ± SEM. [a]P<0.01 and [b]P<0.001 vs. CCl$_4$; [c]P<0.001 vs. control. AST, aspartate transaminase; ALT, alanine transaminase; CCl$_4$, carbon tetrachloride.

and bands were quantified using the ImageJ software (version 2.0.0; National Institutes of Health).

Evaluation of serum TGF-β and interleukin-6 levels. Commercially available ELISA kits (Cusabio Biotech Co., Ltd.) were used to measure serum levels of TGF-β (cat. no. CSB-E04726m) and IL-6 (cat. no. CSB-E04639m) according to manufacturer's protocol.

Biochemical analysis. Isolated sera were used for the assessment of serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST) (cat. no. AT-1034; Bio-diagnostic), albumin (cat. no. AB-1010; Bio-diagnostic), bilirubin (cat. no. BR-1110; Bio-diagnostic), lactate dehydrogenase activity (LDH; cat. no. 278001; Spectrum Diagnostics), total cholesterol (cat. no. CH-1220; Bio-diagnostic) and triglycerides (TG; cat. no. TR-2030; Bio-diagnostic). Liver homogenates were used for estimating levels of lipid peroxides (measured as malondialdehyde, MDA; cat. no. MD-2529; Bio-diagnostic), catalase activity (cat. no. CA-2517, Bio-diagnostic) and total anti-oxidant activity (cat. no. TA-2513, Bio-diagnostic). Commercially available kits were used for measuring the different biochemical parameters according to the manufacturers' protocols.

Histopathology. Freshly isolated liver specimens were first formalin-fixed (10% w/v in PBS, overnight at 4˚C) then embedded in paraffin following dehydration in a series of increasing ethanol concentrations. The tissues were then cut into 5 µm-thick sections, de-paraffinized, rehydrated and stained with hematoxylin for 7 min and eosin for 1 min at room temperature (H&E staining) as well as Masson's trichrome (19) or immunostained against COX-2 using a specific COX-2 antibody according to the method described by Wójcik *et al* (20). An independent pathologist blindly estimated the degree of liver damage using an Optika B-810 microscope (Optika SRL). Knodell index was used for scoring the degree of liver damage as follows: 0, absence of fibrosis; 1, portal fibrosis; 2, fibrous portal expansion; 3, bridging fibrosis (portal-portal or portal-central linkage); and 4, cirrhosis (21).

High-performance liquid chromatography (HPLC) and characterization of BS extract. BS oleo-gum resin used in the present study was previously characterized by HPLC (16,22,23). It was found to be a combination of α- and β-boswellic acids and their derivatives, including 3-O-acetyl-α-boswellic acid, 11-keto-β-boswellic acid (AKB) and 3-O-acetyl-11-keto-β-boswellic acid (AKBA). Based on the data obtained from the provider, the actual concentrations of KBA and AKBA in the extract were 5.48 and 4.66%, respectively.

Statistical analysis. Statistical analysis was performed using GraphPad Prism (version 7; GraphPad Software, Inc.). Data were presented as mean ± standard error of mean (SEM). Comparisons were performed using one-way ANOVA followed by Tukey-Kramer multiple comparisons test. Results of histopathological analyzes were performed using non-parametric Kruskal-Wallis test followed by Bonferroni's correction. P<0.05 was considered to indicate a statistically significant difference.

Results

Effects of BS on the activities of serum liver enzymes. The levels of ALT, AST and LDH were evaluated in the serum from different test groups as markers of hepatocyte integrity. CCl$_4$ treatment resulted in significant increases in serum ALT and AST activities compared with healthy control animals (P<0.001; Table I). Daily treatment with BS (150 mg/kg) significantly reversed the elevated serum levels of these three biomarkers induced by CCl$_4$ (P<0.001; Table I). The values obtained after BS treatment were comparable to healthy control as well as silymarin treated group (Table I).

Effects of BS on serum cholesterol, TG, albumin and bilirubin levels. CCl$_4$ administration resulted in significant increases in the serum levels of cholesterol, TG and bilirubin (P<0.001) along with a significant drop in albumin levels (P<0.001; Table I), effects that were significantly reversed by subsequent BS administration (P<0.001; Table I).

Effects of BS on total antioxidant capacity and catalase activity. The hepatic tissue antioxidant capacity and catalase activity were then measured in the four test groups. A significant reduction in catalase and total antioxidant activities were observed
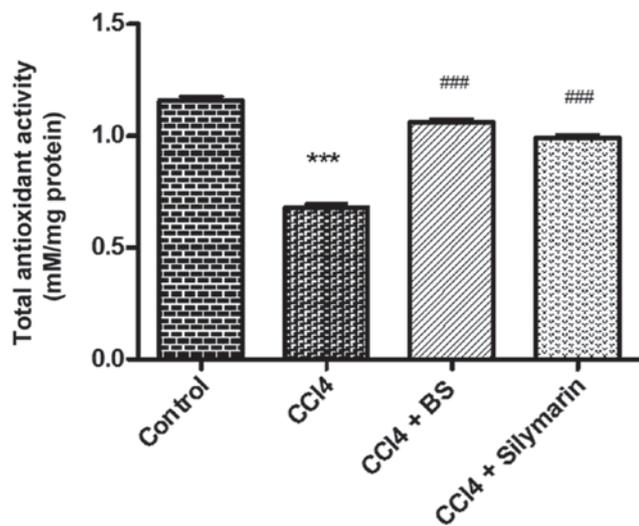
Figure 1. Total antioxidant activity (mM/mg protein) as measured in liver homogenates from the four test groups. A significant reduction in total antioxidant activity in hepatic tissues was observed following CCl_4 administration compared with healthy control. BS administration significantly improved CCl_4-induced inhibition of total antioxidant activity in an effect that was similar to silymarin. Each value represents the mean ± SEM with 8 rats/group. ***P<0.001 vs. Control and ###P<0.001 vs. CCl_4. CCl_4, carbon tetrachloride; BS, *Boswellia serrata* gum extract.
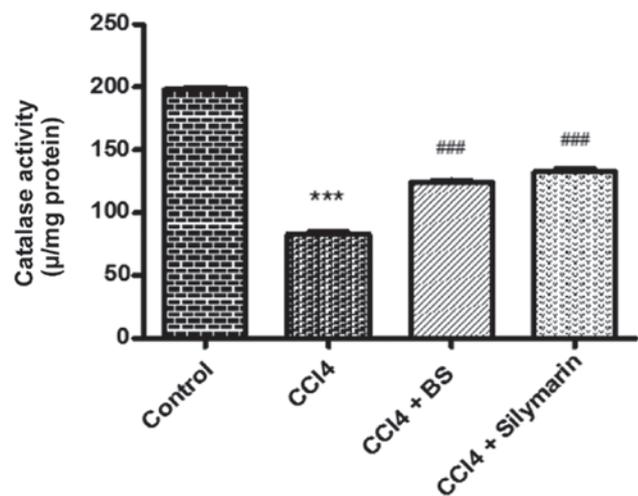

Figure 2. Catalase activity (units/mg protein) in liver homogenates from the four test groups. Catalase activity in hepatic tissues showed a significant reduction upon CCl_4 administration compared with healthy control. BS administration significantly ameliorated CCl_4-induced inhibition of catalase activity in an effect that was similar to silymarin. Each value represents the mean ± SEM with 8 rats/group. ***P<0.001 vs. Control and ###P<0.001 vs. CCl_4. CCl_4, carbon tetrachloride; BS, *Boswellia serrata* gum extract.

in the CCl_4 group compared with normal control (P<0.001, Figs. 1 and 2). Treatment with BS or silymarin resulted in significant increases in catalase and total antioxidant activity compared with the CCl_4 group (P<0.001; Figs. 1 and 2).

Effects of BS on lipid peroxidation in hepatic tissues. For the present study, malondialdehyde (MDA) levels in hepatic tissues were used as a marker for lipid peroxidation. CCl_4 treatment induced significant increases in tissue MDA levels compared with normal control (P<0.001; Fig. 3). BS treatment resulted in a significant reduction in the elevated MDA levels compared with the CCl_4-treated group (P<0.001; Fig. 3), to levels that were comparable to those of healthy control and the silymarin-CCl_4-treated group.

Effects of BS on the expression and transcription levels of nuclear NF-κB and TNF-α. Changes in nuclear NF-κB p65 protein expression and TNF-α transcription in liver tissues from the four treatment groups were next measured to assess the anti-inflammatory activity of BS (Fig. 4A and B). CCl_4 treatment resulted in an increase in the expression of the pro-inflammatory markers, TNF-α mRNA and NF-κB proteins, compared with healthy controls. BS administration reversed this CCl_4-induced effect to levels comparable with control and similar to that induced by silymarin treatment (Fig. 4A-D).

Effects of BS on the expression levels of TGF-β and interleukin-6. To evaluate the effect of different treatments on the expression of inflammatory markers, the levels of the pro-inflammatory markers TGF-β and IL-6 were measured in the serum from the four treatment groups. CCl_4 treatment significantly increased the serum levels of both markers as a result of the induced inflammatory response (Fig. 5A and B).
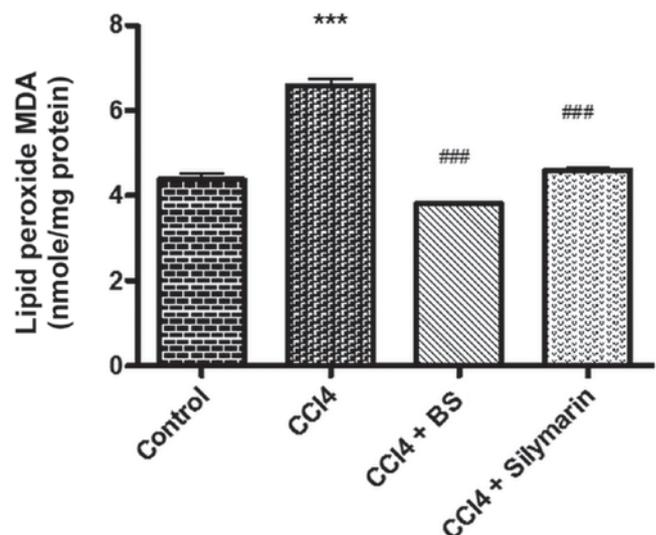

Figure 3. Malondialdehyde (nM/mg protein) levels in liver homogenates from the four test groups. Using malondialdehyde levels as marker, lipid peroxidation in hepatic tissues increased significantly following CCl_4 treatment compared with healthy control. BS administration significantly alleviated CCl_4-induced lipid peroxidation to levels similar to that produced by silymarin. Each value represents the mean ± SEM with 8 rats/ group. ***P<0.001 vs. control group and ###P<0.001 vs. CCl_4. MDA, malondialdehyde; CCl_4, carbon tetrachloride; BS, *Boswellia serrata* gum extract.

Subsequent treatment with BS partially but significantly reversed the increased expression of TGF-β and IL-6 to values comparable to those obtained after silymarin treatment (Fig. 5A and B).

Histopathological analysis. Liver sections from the four treatment groups were subsequently stained against COX-2 to investigate the extent of inflammation. Upon CCl_4 treatment, strong COX-2 expression was detected in infiltrated mononuclear phagocytes compared with control sections
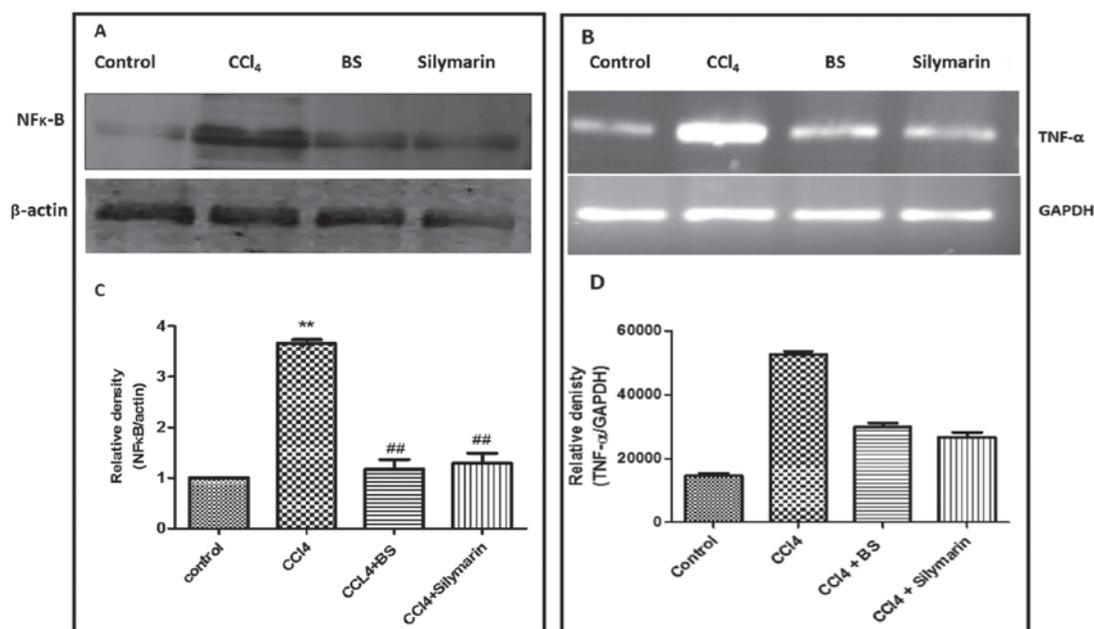
Figure 4. Effect of BS treatment on the expression of nuclear NF-κB and TNF-α levels in liver tissues from the four test groups. (A) Western blot analysis of nuclear NF-κB protein expression using β-actin as an internal loading control. (B) Reverse-transcription-semi quantitative PCR analysis of TNF-α mRNA expression using GAPDH as the internal loading control. A significant increase in NF-κB as well as TNF-α levels was observed after CCl$_4$ treatment which was significantly reversed after BS treatment. (C) Quantified densitometry analysis of (A). (D) Quantified densitometry analysis of (B). Each value represents mean ± SEM for 8 rats/group. ***P<0.001 vs. Control and ###P<0.001 vs. CCl$_4$. CCl$_4$, carbon tetrachloride; BS, *Boswellia serrata* gum extract; TNF-α, tumor necrosis factor-α.
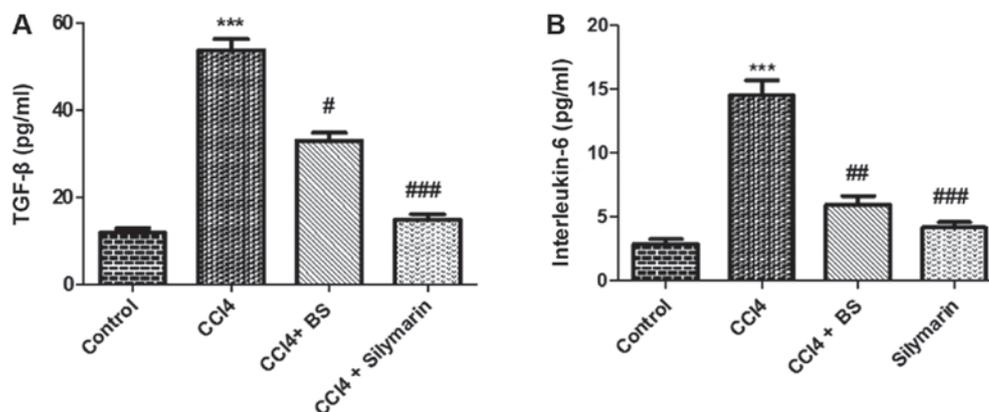

Figure 5. Expression of TGF-β and IL-6 in the serum of the four treatment groups. (A) CCl$_4$ treatment resulted in a significant increase in the serum levels of TGF-β and (B) IL-6 compared with healthy controls. Administration of BS or silymarin after CCl$_4$ treatment partially but significantly restored the serum levels of both markers compared with CCl$_4$. TGF-β, transforming growth factor-β; IL-6, interleukin-6; CCl$_4$, carbon tetrachloride; BS, *Boswellia serrata* gum extract.

(Fig. 6A and B). This expression was ameliorated following BS or silymarin treatment (Fig. 6C and D), suggestive of their curative effects.

Examination of liver sections stained using H&E reflected normal morphology and architecture in the control group (Fig. 7A). In contrast, extensive hepatocellular damage including portal inflammation, venous congestion, and fatty changes in the form of hepatocyte vacuolization and fatty droplets were observed in liver tissue sections from animals treated with CCl$_4$ (Fig. 7B). BS treatment ameliorated CCl$_4$-induced liver damage, an effect that was similar to that achieved by silymarin treatment (Fig. 7C and D). BS and silymarin treatment significantly reversed CCl$_4$-induced histopathological damage in terms of fibrosis and inflammation scores (P<0.05; Fig. 7E and F).

Masson's trichrome staining was performed to evaluate the extent of liver fibrosis. Liver sections prepared from normal control rats exhibited normal architecture with minimal accumulation of collagen fibers (Fig. 8A). Tissues from rats following the administration of CCl$_4$ showed disrupted tissue architecture, extended collagen fibers, formation of large fibrous septa, separation of the pseudolobe and accumulation of collagen as observed by intense signals of collagen staining (Fig. 8B). BS and silymarin treatment reversed these pathological changes and decreased collagen deposition (Fig. 8C and D).
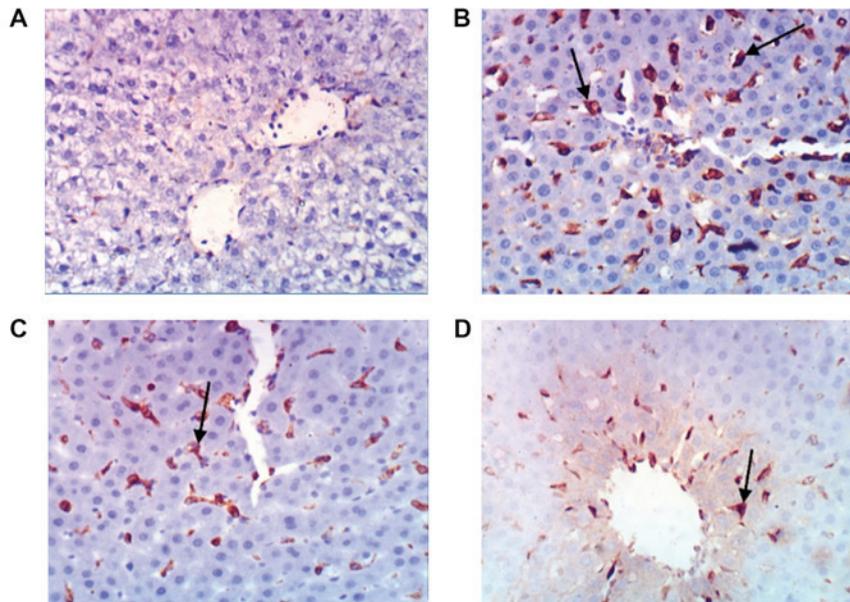
Figure 6. Immunohistochemical staining of COX-2. (A) Liver tissue sections from control animals showing almost no staining for COX-2. (B) Prominent COX-2 expression can be observed in liver tissue sections from the CCl_4-treated group compared with healthy controls. (C) Liver tissue sections from the BS-treated group or (D) the silymarin group exhibited weaker staining for COX-2 compared with the CCl_4-treated group. Arrows indicated COX-2 stained cells. Magnification, x400. COX-2, cyclooxygenase-2; CCl_4, carbon tetrachloride; BS, *Boswellia serrata* gum extract.
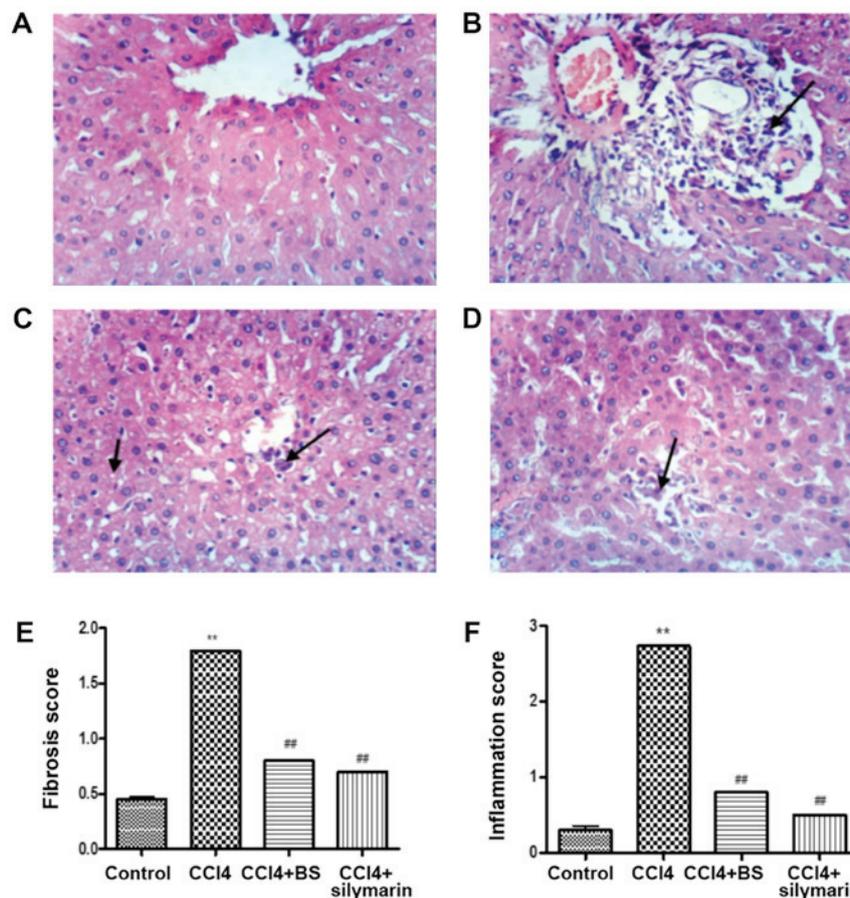

Figure 7. Histological examination of liver sections from the four treatment groups following H&E staining. (A) Liver tissue sections from healthy control show normal architecture of hepatocytes. (B) CCl_4 treatment resulted in damaged cells, shrunken nuclei, portal infiltration with inflammatory cells (arrows), cystic dilatation of bile duct and fibroplasia in the portal triad and centrilobular congestion. (C) BS treatment resulted in restoration of the normal architecture showing Kupffer cells activation and mild vacuolization (arrows) with the absence of congestion. (D) Similar observations as that produced by B.S with limited focal apoptosis (arrows) can be seen in tissues from the silymarin treatment group. Magnification, x400. (E) Quantified fibrosis and (F) inflammation scores. Values represent the mean ± SEM of histopathological scoring. $^{**}P<0.001$ vs. Control and $^{#}P<0.001$ vs. CCl_4. CCl_4, carbon tetrachloride; BS, *Boswellia serrata* gum extract.
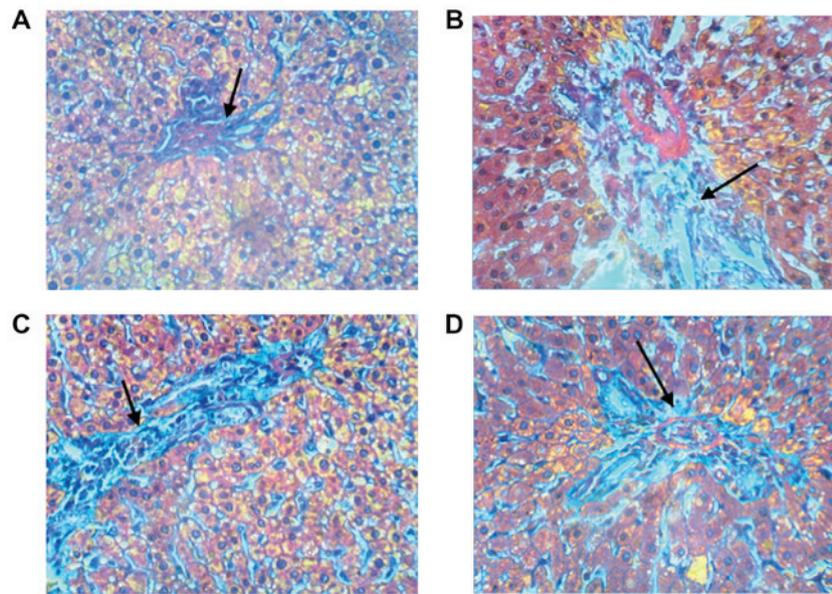
Figure 8. Histological examination of liver sections from the four treatment groups stained with Masson's trichrome. (A) Liver sections from healthy control showed little staining for collagen fibers. (B) Carbon tetrachloride treatment exhibited moderate fibrous septa formation with positive histochemical staining for collagen fibers. (C) *Boswellia serrata* gum extract treatment resulted in marked reductions in collagen fiber bundles, in a similar way to that observed in (D) tissues following silymarin treatment. Magnification, x400. Arrows indicate collagen fibers in the different test groups.

Discussion

In the present study, CCl_4-treated rats exhibited severe inflammatory reactions in the form of increased levels of NF-κB, TNF-α, TGF-β, IL-6, and COX-2 expression. In addition, lipid peroxidation was increased following CCl_4 treatment, which was accompanied with depleted endogenous antioxidant capacity. As a consequence of CCl_4-induced liver damage, hepatocytes lost their membrane integrity and the ability to conjugate or excrete bilirubin, as reflected by the observed elevations in serum bilirubin and liver enzymes levels and activities (ALT, AST and LDH) and disrupted lipid profiles, concordant with a previous report (24).

BS treatment effectively alleviated CCl_4-induced liver injury as it normalized the expression levels of TNF-α and NF-κB. It also reversed signs of the inflammation as reported by the observed normalization of tissue COX-2 expression and serum levels of TGF-β and IL-6. BS treatment reduced lipid peroxidation, recovered antioxidant capacity and increased catalase activity following preceding CCl_4 treatment. BS also normalized lipid profiles, levels and activities of hepatic enzymes and serum bilirubin levels in addition to improving cell integrity.

These effects of BS were comparable to those observed following silymarin treatment, a well-documented hepatoprotective agent. Silymarin consists of a complex of flavonolignans derived from the milk thistle and is famous for its antioxidant, anti-inflammatory, antifibrotic and properties in the liver (25).

Metabolism of xenobiotics such as CCl_4, among other molecules, usually result in the generation of free reactive radicals, a main inducer of hepatocellular injury (26). The hepatotoxic effects of CCl_4 is caused by the intensive oxidative stress generated from its metabolism into highly reactive free radicals (27,28). These free radicals attack several molecules causing lipid peroxidation, loss of membrane integrity, leakage of intracellular enzymes, inflammation and liver injury (17).

Prolonged liver injury leads to the development of fibrosis, cirrhosis and finally hepatocellular carcinoma. However, fibrosis can be reversed by removing the causative agents, including relieving oxidative stress, modifying the inflammatory response, preventing activation of hepatic stellate cells (HSCs) or reducing the production of extracellular matrix (ECM) components, to permit hepatocyte regeneration (29).

However, chronic hepatic injury impairs the regenerative capacity of hepatocytes by activating apoptosis. In response to injury, Kupffer cells (KCs) release cytokines and inflammatory factors, including interleukins, COX-2, TNF-α and TGF-β as reported in the present and a previous study (28). This activates and transforms HSCs into hepatic myofibroblasts, which in turn deposit components of the ECM to initiate fibrosis (30).

TNF-α along with interleukins serve a role in the overexpression of cyclooxygenase-2 and activating the transcriptional factor NF-κB (31). Activated NF-κB amplifies the inflammatory response by activating the transcription of genes involved in the inflammatory response and induces fibrosis by stimulating TGF-β signaling and HSCs activation (32). Cyclooxygnase-2 catalyzes the synthesis of prostanoids that are involved in inflammation, fibrosis and oncogenesis (33).

Membrane integrity of intact liver cells prevents excessive leakage of liver enzymes, including ALT, AST and LDH from the cytosol into the circulation. As membrane integrity becomes lost in response to CCl4-induced hepatotoxicity, liver enzymes escape into the circulation reflecting the severity of the damage (34), and the hepatocytes lose their ability to conjugate or excrete bilirubin, resulting in elevated serum bilirubin levels (24).

Based on the observed antioxidant effects of BS, its administration alleviated oxidative stress leading to diminished cellular damage and improved hepatocyte integrity and functionality. This was evident by the reductions in serum bilirubin and liver enzymes levels, in addition to the downregulation of

cytokines and profibrogenic factors, including TNF-α, NF-κB, IL-6, TGF-β and COX-2. This was confirmed by the positive effects of BS on regenerating liver cells by histological analysis of liver tissue sections.

The antioxidant capacity of natural products is generally associated with the content of polyphenolic compounds in their composition (35). Among other constituents, BS oleo-gum resin is rich in pentacyclic terpenoids that exhibited anti-inflammatory effects by targeting multiple pathways in a range of disorders, including rheumatoid arthritis, ulcerative colitis, bronchial asthma, osteoarthritis and cancer (12). AKBA and boswellic acids have been previously reported to inhibit the activation of NF-κB and production of TNF-α, IL-1, -2, -4 and -6 (7).

Histopathological evaluation of H&E and Masson's trichrome staining showed that CCl$_4$-treatment induced histopathological damage and increased collagen deposition. Treatment with BS resulted in the reduction in fibrosis scoring and achieved marked improvements in reversing these pathological changes. This could be due to the observed ability of BS to recover endogenous antioxidant mechanisms, resulting in the free radicals being scavenged to allow hepatocyte regeneration. This antioxidant effect breaks the vicious cycle of continuous inflammation-fibrosis resulting from the continuous activation of KCs, causing a release of proinflammatory factors, activation of HSCs and the deposition of ECM, aiding fibrosis.

In addition to the low costs on the long-term use, no adverse effects were reported following the use of BS in treating whole rats (36) or colon cancer cell lines (37).

Collectively, these findings lead to the conclusion that BS oleo-gum resin is effective in alleviating CCl$_4$-induced inflammation and fibrosis in the rat liver. This effect may be due to its antioxidant properties and its ability to recover endogenous antioxidant capacity whilst downregulating the inflammatory response. These effects reported in the present study in addition to the lack of reported adverse effects, suggest BS to be a potential therapeutic hepatoprotective agent.

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Authors' contributions

HME performed the western blot analysis and histochemistry, edited and revised the manuscript; MAF performed the biochemical analysis and took part in writing the manuscript; EMM performed animal maintenance, drug administration, interpreted the data and took part in writing the manuscript; MAA took part in writing and revising the manuscript and the statistical analysis of data; AMS provided *Boswellia* and took part in data analysis and verification; MMA designed the study, and revised and edited the manuscript.

Ethics approval and consent to participate

Experiments on animals were performed in accordance with the international ethical guidelines for animal care of the United States Naval Medical Research Centre, Unit No. 3, Abbaseya, Cairo, Egypt, accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International. The present study was approved by The Research Ethics Committee of the Faculty of Pharmacy, Minya University (Minya, Egypt).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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