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Original article

Antioxidant and hepatoprotective effects of carrot seed oil in a cholestasis-induced rat model: involvement of the SIRT1/FOXO1 signaling pathway

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Abstract

Objective: Cholestasis, caused by impaired bile secretion or biliary obstruction, is associated with oxidative stress and liver injury. This study investigated the antioxidant and hepatoprotective effects of carrot (Daucus carota) seed essential oil in a cholestatic rat model, focusing on oxidative stress– and apoptosis-related gene expression.

Materials and methods: Twenty-four adult male Wistar rats were randomly divided into four groups: control, sham-operated, bile duct ligation (BDL), and BDL treated with D. carota seed essential oil (2 mg/kg/day) for 21 days. Blood and liver samples were analyzed using biochemical, histological, immunohistochemical, and molecular methods.

Results: BDL significantly increased serum ALT, AST, ALP, total bilirubin, and oxidative stress markers, including malondialdehyde (MDA) and superoxide dismutase (SOD) ($p<0.001$). RT-PCR analysis showed significant downregulation of antioxidant-related genes, including SIRT1, FOXO1, and Nrf2, in cholestatic rats. Treatment with carrot seed oil significantly restored the expression of these genes while reducing inflammatory mediators, including inducible nitric oxide synthase (iNOS) and NF-κB ($p<0.01$). In addition, cyclooxygenase-2 (COX-2) protein expression was significantly decreased in treated animals compared with untreated BDL rats ($p < 0.001$). Histological findings further demonstrated improvement in hepatic tissue architecture following treatment.

Conclusion: Carrot seed essential oil exerts potent antioxidant and hepatoprotective effects in cholestatic rats through modulation of the SIRT1/FOXO1/Nrf2 signaling pathway and suppression of inflammatory responses, highlighting its therapeutic potential against oxidative stress–related liver injury and fibrosis.

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Introduction

Cholestasis is a pathological condition characterized by impaired bile flow due to hepatocellular secretory dysfunction or obstruction of intrahepatic or extrahepatic bile ducts. Bile mainly consists of bile salts, lipids, electrolytes, and organic anions(Ibrahim et al. 2022). Recent studies have shown that cholestasis contributes to liver inflammation and oxidative stress(Woolbright and Jaeschke 2019). The resulting Reactive Oxygen Species (ROS) can activate fibroblasts, which synthesize extracellular matrix (ECM) proteins including collagen, fibronectin, and elastin, contributing to fibrosis(Tracy et al. 2016).

Daucus carota L. (Apiaceae), commonly known as wild carrot, is a widely cultivated vegetable with a long history of use in traditional medicine(Bradeen et al. 2002). Phytochemical analyses have identified various active constituents in carrot seeds, including volatile oils, steroids, tannins, flavonoids, and carotenoids(Musa Özcan and Chalchat 2007; Sun T, Simon PW 2009) . A Gas Chromatography-Mass Spectrometry (GC-MS) analysis of Iranian carrot seed essential oil revealed that monoterpenes and sesquiterpenes are the major constituents, with carotol comprising 73.70% of the oil (Jasicka-Misiak et al. 2005).These results corroborate previous findings by on the high carotol content of wild carrot seeds(Jasicka-Misiak et al. 2005).

The pathophysiology of cholestatic liver diseases is closely linked to the C-Reactive Protein (CRP)–Cyclooxygenase-2 (COX2)–NF-κB signaling axis. Sirtuin 1 (*SIRT1*) regulates multiple metabolic processes through deacetylation of transcription factors and signaling proteins via interactions with NF-E2-related factor 2 (Nrf2)(Chang HC 2014). It has been demonstrated that the SIRT1 agonist resveratrol significantly inhibits acetylation and phosphorylation of c-JNK and c-FOS, thereby downregulating COX-2 expression(de Gregorio et al. 2020; Alam F 2021; Song Y, Wu Z 2021)Dysregulation of SIRT1 and impaired Nrf2 signaling have been implicated in cholestatic liver injury, whereas upregulation of these pathways contributes to hepatoprotection(Yu et al. 2017; Wei X et al. 2020) Furthermore, SIRT1 activates multiple cytoprotective signaling pathways including Nrf2, AMPK/PGC-α, and FoxO-mediated autophagy(Fontana RJ 2014; Olson et al. 2017). The transcription factor FOXO1 also plays a key role in energy homeostasis and nutrient metabolism and amplifies inflammatory responses via Toll-Like Receptor 4 (TLR4)- and Signal Transducer and activator of transcription 6 (STAT6)-mediated signaling in macrophages(Lee et al. 2022).

The present study aimed to investigate the total antioxidant capacity and hepatoprotective effects of carrot seed essential oil using a bile duct ligation-induced cholestasis model in Wistar rats.

Materials and Methods

Drugs and reagents

General analytical-grade chemicals were obtained from Sigma (USA) and Merck (Germany). Masson's trichrome stain was purchased from Sigma (USA). Ketamine and xylazine were sourced from Alsafan (Netherlands). Primary anti-cyclooxygenase-2 (COX-2) antibodies were obtained from Abcam (UK). Immunoassay ELISA kits (Biorex, UK) and Alkaline Phosphatase (ALP) kit profiles (Delta DP, Iran). cDNA synthesis and real time PCR were performed via the ExcelRTTM Reverse Transcription Kit (SMOBIO, Taiwan) and RealQ Plus 2x Master Mix Green, High ROX™ kit (Ampliqon, Denmark).

Essential oil

Dried carrot seeds (*D. carota* L.) were finely ground into powder and subjected to hydrodistillation for four hours using a Clevenger-type apparatus. The essential oil obtained was then dried over anhydrous

sodium sulfate and stored in amber vials at 4°C until further use(Lee et al. 2022).

Determining the antioxidant capacity of carrot seed essential oil

The antioxidant activity of carrot seed essential oil was determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay (Natanzi and Arab-Rahmatipour 2014).A 100 µM DPPH solution was freshly prepared in 100% methanol. For the assay, 100 µl of the DPPH solution was added to each sample well, followed by incubation at room temperature for 30 min in the dark. The absorbance was measured at 517 nm using a Stat Fax 3200 microplate reader (Awareness Technology Inc., USA). The antioxidant activity was calculated as the percentage of radical scavenging activity (RSA%) using the following formula: RSA%=ABlank-ASampleABlankx100

Where:

A_{Blank}- Absorbance of the control sample (100 % methanol + DPPH)

A_{Sample}- Absorbance of experimental sample (essential oil + DPPH in methanol 100 %).

Experimental design

A total of 24 adult male Wistar rats (weighing 250±20 g) were housed in polypropylene cages under standard laboratory conditions (temperature: 23 ± 2°C; 12 hr light/dark cycle). All experimental procedures were conducted in accordance with ethical guidelines and approved by the Ethics Committee of Kharazmi University, Karaj, Iran (Approval code: IR.KHU.REC.1403.031). Animals were randomly assigned to four experimental groups (n=6 per group): Group 1 was considered the control group, receiving no surgical intervention or treatment; Group 2 was a sham group and underwent a surgical laparotomy without bile duct ligation (BDL); Group 3 BDL-induced obstructive cholestasis via BDL without treatment, and Group 4 received treatment with carrot seed essential oil orally 2 mg/kg /day for 21 days(Kabiri-Arani S 2024).

Surgical procedures

Surgical procedures were meticulously performed, under complete aseptic conditions. Anesthesia was induced via intraperitoneal injection of ketamine (75 mg/kg) and xylazine (5 mg/kg), administered in the same syringe. A mid-line laparotomy was preformed starting from the xiphoid cartilage. Obstructive cholestasis was induced by identifying and double-ligating the common bile duct (1 cm apart) using 5-0 polypropylene suture. Following ligation, the exteriorized organs were repositioned, and the abdominal cavity was rinsed with sterile 0.9% NaCl solution. The abdominal incision was then sutured in layers. Postoperative analgesia was provided with meloxicam (5 mg/kg/day) for three days. An antibiotic regimen with cefotaxime sodium (50 mg/kg/day) was also administered intraperitoneally for three consecutive days to prevent infection.

Blood and liver sampling

Blood samples were collected via cardiac puncture using 18-gauge needles attached to 10 ml syringes, under sterile conditions and without anticoagulants. Samples were centrifuged at 2000 rpm for 10 min to separate serum, which was stored at –20°C until biochemical analysis.

Serum biochemical analysis

Serum samples were analyzed to determine the levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and total bilirubin (TB). These biochemical markers were measured using standard ELISA kits (Biorex, UK) and automated biochemical analyzers. ALP levels were assessed using the ALP kit profile from Delta DP (Iran), and measurements were performed using a biochemistry analyzer (Robonic, India).

Determination of liver malondialdehyde (MDA) and superoxide dismutase activity (SOD)

Liver tissues were homogenized in saline to create a 10% homogenate, followed by centrifugation to obtain a supernatant. This supernatant was diluted to a 1% concentration for subsequent MDA measurement. For the measurement, the diluted supernatant was combined with Trichloroacetic acid (TCA) and Thiobarbituric acid (TBA), then heated to develop color. After cooling, n-butanol was added, shaken, and centrifuged to isolate the pink-colored butanol layer, which was analyzed for absorbance at 532 nm(Al-Serwi and Ghoneim 2015).

For SOD activity, liver tissues were homogenized in phosphate-buffered saline with dithiothreitol and centrifuged to obtain a supernatant. SOD activity was assessed using the Nitro blue Tetrazolium (NBT) reduction method with a specific reaction mixture. The mixture was incubated, and NBT reduction inhibition was measured at 470 nm. SOD activity was quantified as U/mg protein based on inhibition compared to a standard curve(Ismail et al. 2012).

Histopathological examination

Liver tissue samples were fixed in 10% neutral-buffered formalin, dehydrated through a graded ethanol series, and embedded in paraffin wax. Sections of 5 µm thickness were prepared from the paraffin-embedded blocks and stained with Masson's trichrome stain to evaluate collagen fiber distribution and the degree of fibrosis. Histological evaluation was performed on five slides per group, with ten randomly selected fields per slide. Quantification of collagen area (%) was done using ImageJ software in 10 random fields per slide. Fibrosis severity was graded using the Metavir and Ishak system (Mohamadnejad et al. 2010).

RNA isolation and real-time PCR

Total RNA was extracted from liver tissues of control, cholestatic, and treated rats using TRIzol reagent (Invitrogen, USA) following the manufacturer's protocol. RNA quality was confirmed by agarose gel electrophoresis under UV illumination, and concentration was measured spectrophotometrically at 260/280 nm. For cDNA synthesis, 3 µg of total RNA was reverse transcribed at 40°C for 1 hr. using Superscript II reverse transcriptase (Invitrogen, USA). Real-time PCR was conducted using SYBR Green PCR Master Mix (Roche, Pleasanton, CA, USA) in a StepOnePlus™ RT-PCR system (Applied Biosystems, USA). The housekeeping genes used for normalization were *B2M*.

Relative expression levels of target genes (*SIRT1, FoxO1, Nrf2, iNOS, and NF-κB*) were quantified using the 2^-ΔΔCt method. All reactions were performed in duplicate. The primer sequences used are listed in Table 1.

Table 1. The primer sequences.

Gene	Forward Primer (5'-3')	Reverse Primer (5'-3')
SIRT1	+GCAACAGCATCTTGCCTGAT	-GTGCTACTGGTCTCACTT
Fox1	+CATACACAGATGCACGCACG	-GGTACGGCATACGGGTACAG
Nrf2	+CCGGTGACAGGCTTTTTGTG	-AGGTTACCCTAGAGGCCAGG
iNOS	+CAGGAAAATCCCTGGACCCC	-CTGCACCACACAAAGTGAGC
NF-κB	+AGGACGTGGAGTCAGGCTAT	-AGGAACCCATACTTGCAGGC

Immunohistochemical examination

Liver tissues were fixed in formalin for immunohistochemical analysis, with antigen retrieval performed using citrate buffer and microwave heating. Sections were washed with Phosphate Buffer Saline (PBS) and maintained in a humidity chamber during staining, with tissue regions outlined for clarity. To prevent nonspecific binding, sections were incubated with a blocking solution, rinsed with PBS, and treated with a primary

antibody against COX-2 overnight. After further washes, a secondary antibody was applied, followed by the streptavidin-peroxidase complex and additional PBS washes. Immunostaining was visualized using 3,3′-diaminobenzidine (DAB), with nuclear counterstaining performed using hematoxylin. Slides were then prepared for analysis, and COX-2 expression was evaluated microscopically, with staining intensity quantified using ImageJ software(Karimzadeh et al. 2013). Semi-quantitative immunohistochemistry (IHC) analysis of COX-2 expression was performed using a complementary approaches: Area-based scoring (H-score): The proportion of area cells with weak (1+), moderate (2+), and strong (3+) cytoplasmic staining was estimated. H-score was calculated as: H-score = (% weak ×1) + (% moderate ×2) + (% strong ×3), yielding a score between 0 and 300.

Statistical analysis

Data analysis was conducted using GraphPad Prism version 9.0 (GraphPad Software, USA). One-way analysis of variance (ANOVA) followed by Tukey's post hoc test was used for comparisons among multiple groups. When appropriate the Kruskal-Wallis test was applied for nonparametric data. All data are expressed as mean ± standard deviation (SD). A p-value of less than 0.05 was considered significant.

Results

Antioxidant properties

The antioxidant activity of *D. carota* seed essential oil was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay and compared to ascorbic acid as a standard. The experiment was conducted in duplicate. Results demonstrated that the essential oil exhibited significant free radical scavenging capacity, with a mean IC_{50} value of 12.5 µg/ml, compared to 38.63 µg/ml for vitamin C.

The DPPH reduction was assessed by measuring absorbance at 517 nm. Based on the DPPH assay, the IC_{50} value of the essential oil ranged between 10 and 15 µg/ml, indicating stronger antioxidant potential than the reference compound, ascorbic acid (IC_{50} = 38.63 µg/ml) ($p \leq 0.01$) (Figure 1).

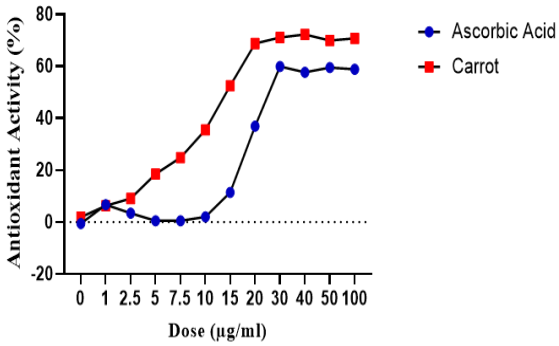

Figure 1. The DPPH scavenging activity of the *Daucus carota* extract at different concentrations

Evaluation of liver enzymes

Following three weeks of Bile Duct Ligation (BDL), serum liver enzyme levels were significantly elevated in the cholestasis group compared to the control and sham groups. One-way ANOVA analysis indicated a marked increase in the activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and total bilirubin (TB) ($p < 0.001$).

Oral administration of carrot seed essential oil (2 mg/kg/day) to BDL rats resulted in a significant reduction in the levels of these hepatic biomarkers compared to the untreated BDL group ($p < 0.05$ to $p < 0.001$). This hepatoprotective effect was particularly evident in the reduction of ALT and AST levels (Figure 2).

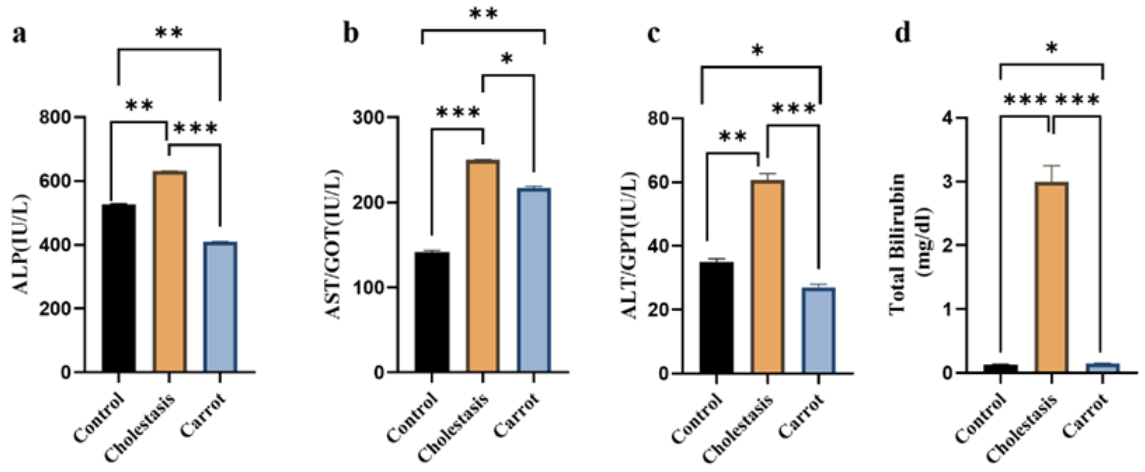

Figure 2. Serum markers of liver damage alkaline phosphatase (ALP) (a), aspartate aminotransferase (AST) (b), alanine aminotransferase (ALT) (c) and total bilirubin (d) levels. Data are presented as means ± SD (n = 6 per group). A p value of ≤ 0.05 was considered significant, *p<0.05, **p<0.01, and ***p<0.001.

Malondialdehyde (MDA) content and superoxide dismutase (SOD) activity in liver

Three weeks after bile duct ligation, oxidative stress markers were assessed in liver tissue. One-way ANOVA revealed that MDA levels were significantly elevated in the cholestasis group compared to the control group ($p < 0.001$). However, rats treated with carrot seed essential oil (2 mg/kg/day) exhibited a substantial reduction in MDA levels compared to both the untreated BDL group and the control group ($p < 0.01$), indicating a strong antioxidant effect.

Regarding enzymatic antioxidant activity, SOD levels were also significantly decreased in the cholestasis group ($p < 0.01$), suggesting an adaptive response to oxidative stress. Treatment with carrot seed oil resulted in a notable increase in SOD levels compared to the untreated cholestasis group ($p < 0.05$), approaching values observed in the control group (Figure 3).

Histopathological examination: Masson's trichrome staining

Masson's trichrome staining was performed to assess collagen deposition and fibrosis in liver tissues. In the cholestasis group, a significant increase in collagen content was observed compared to the control group. In contrast, liver sections from rats treated with carrot seed essential oil (2 mg/kg/day) exhibited a notable reduction in collagen accumulation, with preservation of hepatic architecture and minimal fibrotic expansion. Quantitative analysis using ImageJ software confirmed a significant decrease in collagen fiber percentage in the treatment group compared to the untreated cholestasis and sham-operated groups (Table 2 and Figure 4).

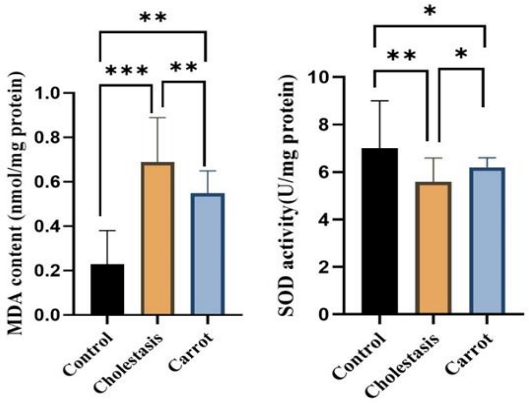

Figure 3. Liver markers of MDA (a) and SOD (b) levels. Data are presented as means ± SD (n = 6 per group). A p value of ≤ 0.05 was considered significant. *p<0.05, **p<0.01, and ***p<0.001.

Table 2. Fibrosis scoring base on Metavir and Ishak systems.

Group	Metavir Score (F0–F4)	Ishak Fibrosis (0–6)
Control	F0	0
Sham	F0	0–1
Cholestasis	F3	4
Carrot	F1–F2	2–3

RNA isolation and real-time PCR

RT-PCR analysis revealed a significant decrease in the expression of *SIRT1*, *FoxO1*, and *Nrf2* genes in cholestatic rats compared to the control and carrot seed oil-treated groups (*SIRT1* and *FoxO1*: $p < 0.001$; *Nrf2*: $p < 0.01$). Conversely, a significant increase was observed in the expression of *iNOS* and *NF-κB* in the BDL group ($p < 0.01$), indicating upregulated inflammatory and oxidative stress responses.

Treatment with carrot seed essential oil (2 mg/kg/day) significantly reversed these alterations: *SIRT1*, *FoxO1*, and *Nrf2* expression levels were markedly up-regulated, while *iNOS* and *NF-κB* expression was significantly reduced compared to the cholestasis group ($p < 0.01$) (Figure 5).

Evaluation of liver protein expression by immunohistochemistry (IHC)

Semi-quantitative evaluation of COX-2 immunostaining demonstrated a marked increase in the hepatic expression of COX-2 protein in the cholestasis group compared to the control group. COX-2 staining was notably intensified, indicating elevated inflammatory response associated with bile duct ligation. In contrast, treatment with carrot seed essential oil (2 mg/kg/day for 21 days) significantly reduced COX-2 expression in liver tissues. Both H-score and area-based immunoreactivity scoring observed in the treated group was markedly lower than that in the untreated BDL group, indicating a potential anti-inflammatory effect of the essential oil (Figure 6).

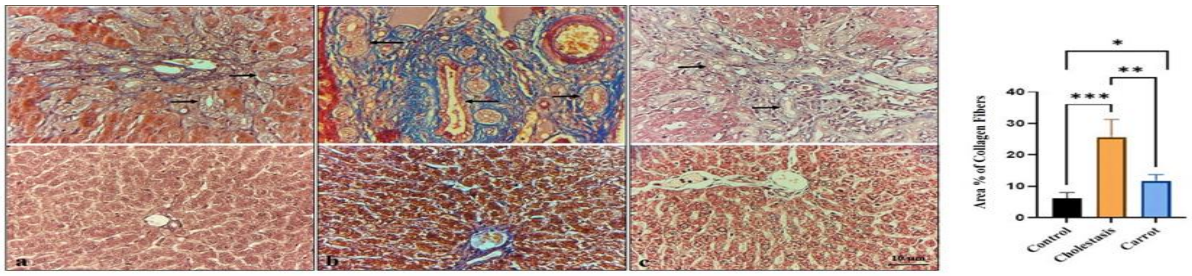

Figure 4. Histopathological analysis of liver sections (Masson's trichrome staining). (a) Control group: Normal hepatic architecture with typical hepatocyte morphology and minimal collagen deposition. (b) Cholestasis group: Exhibits marked periportal fibrosis (blue staining) and prominent bile duct hyperplasia (arrowhead). (c) Carrot seed oil-treated group: Demonstrates significant attenuation of collagen accumulation compared to the cholestasis group. Note the distinct improvement in hepatic histoarchitecture following treatment. Data are presented as means ± SD. (n = 6 per group). A p value of ≤ 0.05 was considered significant. *p<0.05, **p<0.01, and ***p<0.001. The scale bar indicate 50 µm in all panels which were taken at x200 magnification.

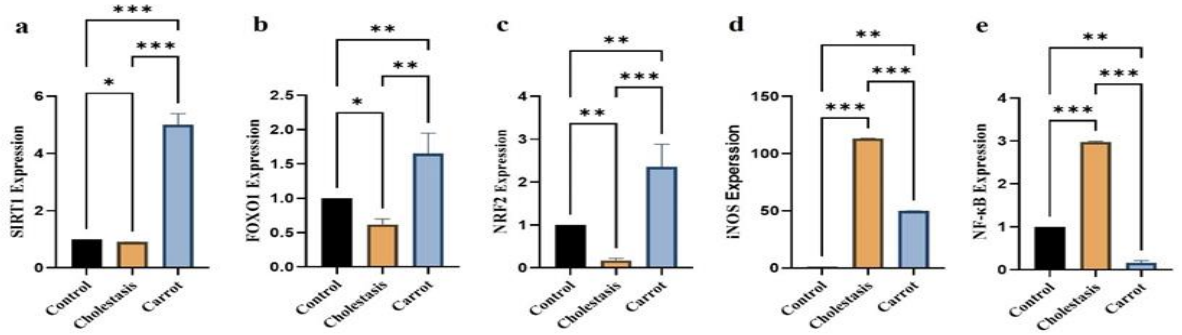

Figure 5. The expression of genes a. *SIRT1*, b. *FoxO1*, and c. *Nrf2*, d. *iNOS* , e. *NF-κB* in liver fibrotic tissue was studied in a cholestasis group, control group, and a group treated with carrot seed oil (*Daucus carota* seed essential oil, 2 mg/kg B.W. /day for 21 days). Carrot seed oil treatment reduced gene levels in cholestasis group, but increased levels in the treated group compared to controls and cholestasis group. BW; body weight. n=6, The data are presented as the means ± SD. Statistical significance was established at a level of p<0.05. *p<0.05, **p<0.01, and ***p<0.001.

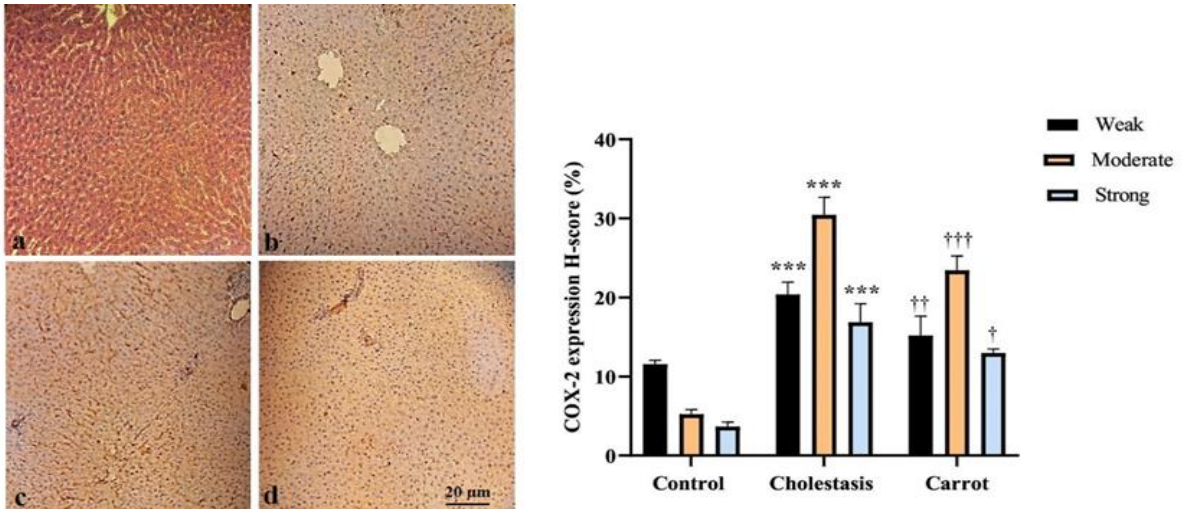

Figure 6. **:** Immunohistochemical staining of liver tissues for COX-2 expression (2 mg/kg B.W. /day for 21 days). (a) Control group, (b) Cholestasis group, (c) Carrot seed oil-treated group. (scale bar: 20 µm). BW; Body Weight. n=6, The data are presented as the means ± SD. *; significant differences compared to the Cholestasis vs. Control, †; comparison of Carrot vs. Cholestasis group. Statistical significance was established at a level of $p<0.05$. *$p<0.05$, **$p<0.01$, and ***$p<0.001$. The scale bar indicate 20 µm in all panels which were taken at x100 magnification.

Discussion

In this study, general analytical-grade reagents and standardized methods were employed to evaluate the antioxidant, biochemical, histopathological, and gene expression effects of carrot seed essential oil in a rat model of cholestasis. The treatment significantly improved antioxidant capacity, reduced liver enzyme levels, fibrosis, and oxidative stress markers (MDA and SOD), and modulated gene/protein expression levels (*SIRT1*, *Nrf2*, and COX-2) compared to the untreated cholestasis group. These findings suggest a protective role for carrot seed oil against BDL-induced liver injury.

Cholestasis impairs this flow, leading to toxic accumulation of bile acids and bilirubin, oxidative stress, and hepatocellular damage. This process triggers the release of ALP, ALT, and AST into the bloodstream, and elevated serum bilirubin and bile acids are key diagnostic markers(Ruiz ARG et al. 2021)(Williamson and Chapman 2015).

The oxidative defense system neutralizes ROS and free radicals through enzymatic pathways modulated by nutrients and non-nutrients(Sharifi-Rad et al. 2020). Natural compounds such as plant-derived antioxidants enhance these defenses. Chemical analyses of carrot seed essential oil consistently identify carotol—a sesquiterpene alcohol— as its predominant component(Sieniawska et al. 2016). dietary carrot seed and turmeric extracts enhanced antioxidant defenses and reduced lipid peroxidation in rats(Rezaei-Moghadam et al. 2012). Consistent with Şehirli et al. (2008), our data showed significantly higher MDA levels in cholestatic liver tissues ($p< 0.001$), indicating severe oxidative stress and lipid peroxidation, while SOD activity declined. These biochemical changes correlated with fibrosis severity and confirmed the oxidative stress-mediated liver damage.

Additionally, the antioxidant potential of *D. carota* L. seed aqueous extract in hyperlipidemic rats subjected to Triton-induced oxidative stress was investigated and revealed that the carrot seed extract significantly enhanced the antioxidant defense system while counteracting the oxidative and inflammatory effects induced by Triton(Tijjani et al. 2020). Another study examined the antioxidant properties of carrot seed alcoholic extract in male rats exposed to H_2O_2-induced oxidative stress,

demonstrating the extract's ability to prevent oxidative damage(Al-Saadi et al. 2019).

Patients with extrahepatic cholestasis and nonalcoholic fatty liver disease (NAFLD) have been reported to exhibit dysregulated deregulated *SIRT1* levels and activity(Tan M 2015), a phenomenon also observed in various rodent models of liver diseases. Studies have shown that *SIRT1* overexpression and the use of its activators exert protective effects against liver injury, including cholestatic liver damage(Wu et al. 2018).In the current study, we observed a significant upregulation ($p<0.001$) of SIRT1 gene expression in rats treated with carrot seed essential oil compared to those in the control and cholestasis groups.

Studies have demonstrated that genetic deletion of *Nrf2* in mice results in cholestatic symptoms, highlighting its critical role in liver protection(Chen P 2015). A recent study has demonstrated that BDL, as a well-established model of extrahepatic cholestasis, leads to the upregulation of multiple Nrf2-regulated genes. Findings suggest a functional interplay between FOXO1 and SIRT1, as they cooperate to regulate key biological processes including aging and oxidative stress response(Xiong et al. 2011).

Nitric oxide (NO) has multiple and varied functions in the liver. While lower amounts of NO correlate with increased bile flow, higher amounts of NO are linked to cholestasis and liver cell damage(Schonhoff et al. 2011). Kim et al. reported that α-pinene (a key component of carrot seed essential oil) exerts anti-inflammatory effects by suppressing the Nuclear Factor-Kappa B (*NF-κB*) pathway and resulting in the downregulation of Nitric Oxide (NO), Tumor Necrosis Factor-alpha (TNF-α), and interleukin-6 (IL-6) levels, *COX-2*(Kim et al. 2015). A study in activation of *NF-κB* and its associated genes like *COX-2* was inhibited by beta-carotene(Geeviman et al. 2018). Beta-carotene has also been shown to inhibit *NF-κB* activation and normalize *COX-2* levels in the liver(Geeviman et al. 2018; Josson Akkara and Sabina 2020; Matos et al. 2021), further supporting the hepatoprotective and anti-inflammatory effects observed in this study(Josson Akkara and Sabina 2020; Matos et al. 2021). A potential limitation of this study is the evaluation of only a single dose of *D. carota* seed essential oil. This approach was intentionally selected to comply with the ethical principle of Reduction. The median lethal dose (LD_{50}) of this substance has already been rigorously established in previous studies (Šeregelj et al., 2020). As a result, further dose-determination experiments would be redundant and ethically unjustifiable, as they would involve unnecessary animal use. In addition, a comprehensive phytochemical characterization of the *D. carota* seed essential oil used in this study is reported in a parallel investigation currently under review. Therefore, the present study omits compositional analysis and focuses exclusively on evaluating the antioxidant capacity of Daucus carota seed essential oil.

This study systematically investigated changes in antioxidant gene expression in cholestatic Wistar rats treated with *D. carota* seed essential oil. Notably, the observed therapeutic effects may extend beyond hepatic protection, potentially contributing to the alleviation of cholestasis-associated cognitive impairments through the reduction of liver inflammation. These results highlight the potential of carrot seed essential oil as a promising therapeutic agent for managing cognitive and hepatic complications in liver diseases, including cholestasis.

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Conflicts of interest

The authors declare no conflict of interests.

Funding

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Ethical Considerations

There are no “human subjects” in this study. All ethical considerations were taken into account following the Helsinki Convention and the observance of animal rights, and experiments were performed after the approval of the Ethics Committee of the University of Kharazmi (Ethical Code IR.KHU.REC.1403.031.).

Code of Ethics

IR.KHU.REC.1403.031

Availability of data and materials

Data and materials are available upon written request to the corresponding author.

Declaration of conflicting interests

The authors have declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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