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Protective Effects of *Bunium persicum* Essential Oil against Ischemia/Reperfusion-Induced Pancreatic Injury in Rats

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Abstract

Background & Objectives: This study aimed to evaluate the therapeutic potential of *Bunium persicum* (black cumin) essential oil for preventing ischemia/reperfusion (I/R)-induced pancreatic injury in a rat model.

Materials & Methods: The total phenolic and flavonoid contents of the essential oil were determined using the Folin-Ciocalteu and aluminum chloride colorimetric assays, respectively. Antioxidant capacity was assessed using the DPPH radical-scavenging assay, and the chemical composition was analyzed by GC-MS. Pancreatic injury was induced by ischemia followed by reperfusion. Rats received pretreatment once daily for 7 days, administered by oral gavage or intraperitoneal injection, and post-treatment 1 day after the surgical procedure. *Bunium persicum* essential oil was administered at a dose of 0.1 mL/kg. Sham-operated animals underwent laparotomy and ischemia without treatment. The protective effects were evaluated through histological, biochemical, and molecular assessments.

Results: A nonsignificant decrease in the expression of the anti-apoptotic gene *Bcl-2* was observed in rats with I/R-induced pancreatic injury, a finding that correlated with the observed pathological and biochemical alterations. Our data indicate that 7-day oral pretreatment with *Bunium persicum* attenuated I/R-induced pancreatic damage. This protective effect may be associated with the antioxidant properties of the essential oil, as indicated by a nonsignificant change in malondialdehyde (MDA) levels and a significant increase in superoxide dismutase (SOD) activity relative to the positive control.

Conclusion: Oxidative stress plays a key role in the pathophysiology of I/R-induced pancreatic injury. The underlying protective mechanisms of *Bunium persicum* appear to be multifactorial. These findings suggest that the antioxidant constituents of *Bunium persicum* essential oil may help mitigate pancreatic tissue damage, warranting further investigation of its potential therapeutic applications.

Keywords: black cumin, ischemia/ reperfusion injury, pancreatitis, malondialdehyde.

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Introduction

Acute pancreatic injury has been associated with several etiological factors, among which hypotension and the resulting pancreatic hypoperfusion are particularly important. Pancreatic ischemia/reperfusion (I/R) injury, which is inevitable during pancreatic transplantation and may also occur

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in conditions such as hemorrhagic shock and cardiac surgery, is a recognized cause of acute pancreatic insufficiency. I/R-induced tissue injury is characterized by the generation of reactive oxygen species (ROS), dysregulation of intracellular and mitochondrial calcium homeostasis, microvascular dysfunction, incomplete reperfusion of the microcirculation, and an amplified inflammatory response accompanied by leukocyte infiltration (1, 2).

One of the complications of pancreatic ischemia is acute pancreatitis, which is associated with elevated pancreatic enzyme levels, increased interstitial edema, and infiltration of inflammatory cells into pancreatic tissue. Some evidence suggests that the pancreatic parenchyma becomes hypoperfused during acute pancreatitis (3). The microscopic effects of I/R in the pancreas and other organs have been investigated (4). Hoffmann described the pathophysiology and histological changes associated with pancreatic I/R injury (5). Among the most prominent microscopic features of pancreatic I/R injury is apoptosis, which is largely mediated by ROS. Accordingly, antioxidant-based therapeutic strategies aimed at interrupting oxidative chain reactions have attracted considerable attention (6). Dembinsky et al. investigated the effect of grapefruit seed extract on acute pancreatitis induced by I/R in rats and suggested that its protective effect may be attributed to the activation of antioxidant mechanisms and the improvement of pancreatic blood flow (7). In addition, the therapeutic effects of several agents, including melatonin, ghrelin, U-74389G, warfarin, and obestatin, against I/R-induced pancreatitis have been reported (8). Many spices and herbs contain antioxidants that protect cells against oxidative damage. Spices have also been reported to possess anticancer and anti-inflammatory properties, which are often mediated by the inhibition of lipid peroxidation and prostaglandin synthesis (9).

Black cumin (*Bunium persicum* Boiss., BC) is a perennial plant belonging to the family Apiaceae. In traditional and modern medicine, it is used as a carminative, antidiarrheal, antipyretic, cholesterol-lowering agent, antiallergic, hypoglycemic agent, and stimulant of pancreatic enzyme secretion (9). Anti-inflammatory, antimicrobial, antioxidant, free radical-scavenging, and antiparasitic properties have also been reported for this plant (10). Ethnopharmacological interviews have shown that black cumin fruits are traditionally used in Iranian folk medicine to treat spasms, menstrual pain, flatulence, and infections (11).

Essential oils are aromatic oily liquids obtained from various parts of plants (12). The antimicrobial activity of essential oils has been demonstrated in numerous studies. *B. persicum* essential oil contains compounds such as cuminaldehyde, γ-terpinene, and other bioactive terpenes, whose antibacterial and antioxidant activities have been widely investigated (13, 14). Previous studies have emphasized the effects of chemical agents on I/R-induced injury. The present study therefore investigated the efficacy of *Bunium persicum* Boiss. essential oil against I/R-induced pancreatitis.

Materials and Methods

Extraction of *Bunium persicum* Essential Oil

Bunium persicum seeds were obtained from Kerman, Iran, identified, and deposited in the herbarium of Fasa University of Medical Sciences (Voucher No. 100-3). The seeds were dried in the shade at room temperature. The essential oil was extracted from 200 g of seeds by hydrodistillation using a Clevenger-type apparatus for 3 hours. The oil was dehydrated using anhydrous sodium sulfate and stored until further analysis. The chemical composition was determined using gas chromatography-mass spectrometry (GC-MS).

Total Phenolic Content (TPC)

The TPC of the *B. persicum* essential oil

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was determined using the Folin-Ciocalteu colorimetric assay. Briefly, 100 µL of the essential oil (1000 ppm) was mixed with 500 µL of 10% (v/v) Folin-Ciocalteu reagent and incubated at room temperature in the dark for 5 minutes. Subsequently, 400 µL of sodium carbonate (7.5% w/v) was added, and the mixture was incubated for 60 minutes in the dark. The absorbance was measured at 765 nm using a Synergy HTX multimode reader. Gallic acid was used as the standard, and the results were expressed as micrograms of gallic acid equivalents per milligram of dry weight (µg GAE/mg). All measurements were performed in triplicate.

Total Flavonoid Content (TFC)

The TFC was measured using the aluminum chloride colorimetric method. Briefly, 200 µL of the essential oil (1000 ppm) was mixed with 50 µL of sodium nitrite (5% w/v) and 50 µL of aluminum chloride (10% w/v). The mixture was incubated for 5 minutes at room temperature in the dark. After 700 µL of sodium hydroxide (4% w/v) was added to bring the final volume to 1 mL, the solution was stirred and incubated for 15 minutes at room temperature. The absorbance was recorded at 510 nm. Quercetin was used as the standard, and the results were expressed as micrograms of quercetin equivalents per milligram of dry weight (µg QE/mg). All assays were performed in duplicate.

Antioxidant Activity (DPPH Assay)

The total antioxidant capacity was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging assay. Serial dilutions of the essential oil (50, 100, 200, 500, and 1000 ppm) were prepared in 70% ethanol. A 40-µL aliquot of each dilution was then mixed with 160 µL of 0.3 mM DPPH solution and incubated in the dark for 30 minutes at room temperature. The absorbance was measured at 517 nm. Ascorbic acid was used as a positive control. The percentage of radical-scavenging activity (RSA) was calculated as follows:

Antioxidant activity (%) = [(Absorbance of the control − Absorbance of the experimental sample) / Absorbance of the control] × 100

GC-MS Analysis

The chemical composition of the essential oil was analyzed using a TRACE MS system (Thermo Quest-Finnigan, USA) equipped with a DB-5 MS fused-silica capillary column (30 m × 0.25 mm × 0.25 µm). Helium (99.999% purity) was used as the carrier gas at a constant flow rate of 1.1 mL/min. The injector temperature was set at 250 °C. The oven temperature program was as follows: the temperature was increased from an initial 38 °C to 250 °C at a rate of 5 °C/min, followed by a 2-minute isothermal hold. A 0.2 µL sample was injected in split mode (split ratio, 1:100). The relative percentage of each constituent was determined by peak-area normalization.

Experimental Design and Animal Grouping

A total of 49 male Wistar rats (250–300 g) were randomly divided into seven groups (n = 7). Group 1 served as a positive control and received no surgery or treatment. Group 2, the negative control group, received normal saline by oral gavage. The animals in Group 3, the sham-operated group, underwent laparotomy and ischemia without essential oil treatment. Animals in Groups 4–7 underwent laparotomy and ischemia and received BC essential oil at a dose of 0.1 mL/kg (15).

In Groups 4 and 5, the essential oil was administered once daily for 7 days before surgery, either by oral gavage (pre-G+I/R) or by intraperitoneal (*IP*) injection (pre-IP+I/R), respectively. In Groups 6 and 7, the essential oil was administered after surgery, either by oral gavage (post-G+I/R) or by *IP* injection (post-IP+I/R), respectively, 1 day after surgery.

Surgical Procedure

Rats were fasted for 5 hours before surgery. Anesthesia was induced using ketamine (80 mg/kg) and diazepam (15 mg/kg).

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Following a mid-abdominal laparotomy, ischemia was induced in the treatment groups by clamping the gastroduodenal and inferior splenic arteries with atraumatic clips for 60 minutes. Reperfusion was confirmed by removing the clips. Sham-operated animals underwent laparotomy and ischemia without essential oil treatment. After surgery, the abdominal wall was sutured, and the animals were monitored until recovery. All animals, except those in the healthy control group, were euthanized 24 hours after reperfusion for tissue and blood collection.

Histopathological Examination

Pancreatic tissue samples were fixed in 10% formalin, dehydrated in an ascending alcohol series, cleared in xylene, and embedded in paraffin. Sections 4 µm thick were prepared using a rotary microtome (Microm HM 325, Thermo Scientific) and stained with hematoxylin and eosin (H&E). Histopathological changes, including edema, inflammation, necrosis, and hemorrhage, were graded on a scale of 0 to 3, as previously described (8, 15). Morphometric analysis was performed using an Olympus BX-53 microscope equipped with a 1 × 1 mm graticule.

Biochemical Analysis

Serum samples were obtained from the heart 24 hours before surgery and 1 day after surgery, while tissue samples were collected 24 hours postoperatively. Superoxide dismutase (SOD) activity in serum was measured using commercial ELISA kits (ZellBio, Germany). Malondialdehyde (MDA) levels in homogenized pancreatic tissue were evaluated using a specific ELISA kit (Bioassay Technology Laboratory, Cat. No. E0156Ra, China). All measurements were performed using a Synergy HTX multimode reader.

Gene Expression Analysis (RT-qPCR)

Total RNA was extracted from pancreatic tissue using RNXPlus™ reagent (SinaClon, Iran), following the manufacturer's protocol. Complementary DNA (cDNA) was synthesized from total RNA using the RevertAid™ cDNA synthesis kit (Fermentas, Lithuania) in a 20 µL reaction volume. Quantitative real-time PCR (RT-qPCR) was performed using a StepOnePlus™ system (Applied Biosystems, USA) and PCR Master Mix (Fermentas, K0222).

The thermal cycling conditions were as follows: initial denaturation at 95 °C for 5 minutes, followed by 40 cycles of denaturation at 95 °C for 30 s, annealing at 60 °C for *β-actin*, 59 °C for *Bcl-2*, and 62 °C for *p53*, with an additional reported temperature of 58 °C for 40 s, followed by extension at 72 °C for 40 s. Amplification specificity was verified by melt-curve analysis. The relative gene-expression ratio was calculated using the comparative cycle-threshold (Ct) method, with *β-actin* serving as the internal reference gene. Primer sequences, designed using GeneRunner software, are listed in Table 1.

Statistical Analysis

All data are expressed as means ± standard errors of the mean (SEM). Statistical analyses were performed using SPSS software (version 16.0). The normality of the data distribution was assessed using the Shapiro-Wilk test. Differences among groups were evaluated using one-way analysis of variance (ANOVA), followed by Tukey's *post hoc* test for multiple comparisons. Effect size was estimated using partial eta-squared (ηp^2). A *p*-value of ≤0.05 was considered statistically significant.

Table 1. Primer sequences used for RT-qPCR.

Gene	Forward primer (5'–3')	Reverse primer (5'–3')
Bcl2	GATGACTTCTCTCGTCGCTACCGT	ATCCCTGAAGAGTTCCTCCACCAC
p53	CTACTAAGGTCGTGAGACGCTGCC	TCAGCATACAGGTTTCCTTCCACC
β-actin	TTGTGATGGACTCCGGAGAC	ATTGCCGATAGTGATGACCTGA

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Results

Phytochemical Analysis and Antioxidant Activity:

The total phenolic content of *B. persicum* essential oil (BEO) was 23.04 ± 4.41 µg GAE/mg, whereas the total flavonoid content was 76.41 ± 8.82 µg QE/mg. The antioxidant activity of BEO exhibited a concentration-dependent pattern across the concentration range of 50 to 1000 ppm, with significantly greater radical-scavenging activity than the control ($P < 0.0001$). However, the radical-scavenging activity of BEO remained lower than that of the reference antioxidant, ascorbic acid (Table 2, Figure 1). The calculated IC_{50} values were >1000 ppm for BEO and 30.56 ppm for ascorbic acid.

Chemical Composition (GC–MS):

GC-MS analysis identified 16 compounds in BEO, accounting for 99.84% of its total composition (Table 3). The major constituents were γ-p-cymene (2.37%), terpinene (7.07%), α-terpinen-7-al (18.14%), γ-terpinen-7-al (22.54%), and cuminaldehyde (47.61%) (Figure 2).

Histopathological Assessment

All slides were examined for pathological changes in the pancreatic and peritoneal tissues, with particular attention to ischemic necrosis. No pathological changes were observed in the positive and negative control groups. The sham group exhibited multiple necrotic foci accompanied by interlobular edema and hemorrhage in all cases, with reported values ranging from 1.5% to 2.76%. The extent of necrosis was greater in this group than in the other groups. In the *pre-G+I/R* group, only a few small necrotic foci (0.2 to 0.5) were observed in 75% of cases. In the *pre-IP+I/R* group, a few necrotic foci were observed; however, peritonitis was detected in one case. In the *post-G+I/R* group, a few foci of edema and hemorrhage (1%) were observed in 20% of cases. In the *post-IP+I/R* group, a few necrotic foci (5%) were observed in one-third of cases.

Table 2. The antioxidant activity (%) of the Bunium persicum essential oil and ascorbic acid.

CONC (µg/ml)	Bunium persicum		Ascorbic Acid		P-value
	Mean±SD	IC50	Mean±SD	IC50	
50	2.91 ± 1.40	>1000	53.40 ± 3.31	30.56	< 0.0001
100	10.52 ± 0.66		72.15 ± 0.40		< 0.0001
200	10.02 ± 1.28		86.05 ± 0.55		< 0.0001
500	10.75 ± 1.93		89.68 ± 0.24		< 0.0001
1000	15.32 ± 0.84		89.82 ± 0.12		< 0.0001

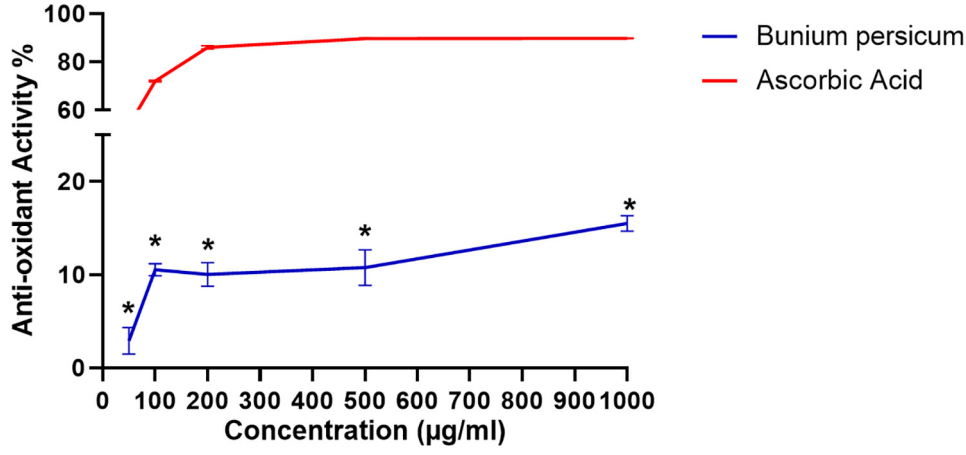


Figure 1. Total antioxidant activity of the Bunium persicum essential oil and Ascorbic Acid. The bullet (*) indicates a statistically significant difference between Bunium persicum and Ascorbic acid.

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Table 3. The phytocomponents of the Bunium persicum essential oil by GC-MS analysis.

No	Compound	Retention time	Area %
1	α-Pinene	4.31	0.04
2	Sabinene	5.06	0.03
3	β-Pinene	5.18	0.1
4	β-Myrcene	5.35	0.02
5	p-Cymene	6.19	2.37
6	Limonene	6.28	0.34
7	1,8-Cineol	6.37	0.03
8	ϒ-Terpinene	6.97	7.07
9	4-Terpineol	10.19	0.13
10	Isogeranial	10.61	0.69
11	Cumin aldehyde	12.07	47.61
12	trans-p-Menth-2-en-7-ol	12.61	0.49
13	Terpinen-7-al <α->	13.19	18.14
14	Terpinen-7-al <γ->	13.3	22.54
15	Mentha-1,4-dien-7-ol <ρ->	14.25	0.18
16	Caryophyllene <(E)->	18.58	0.06

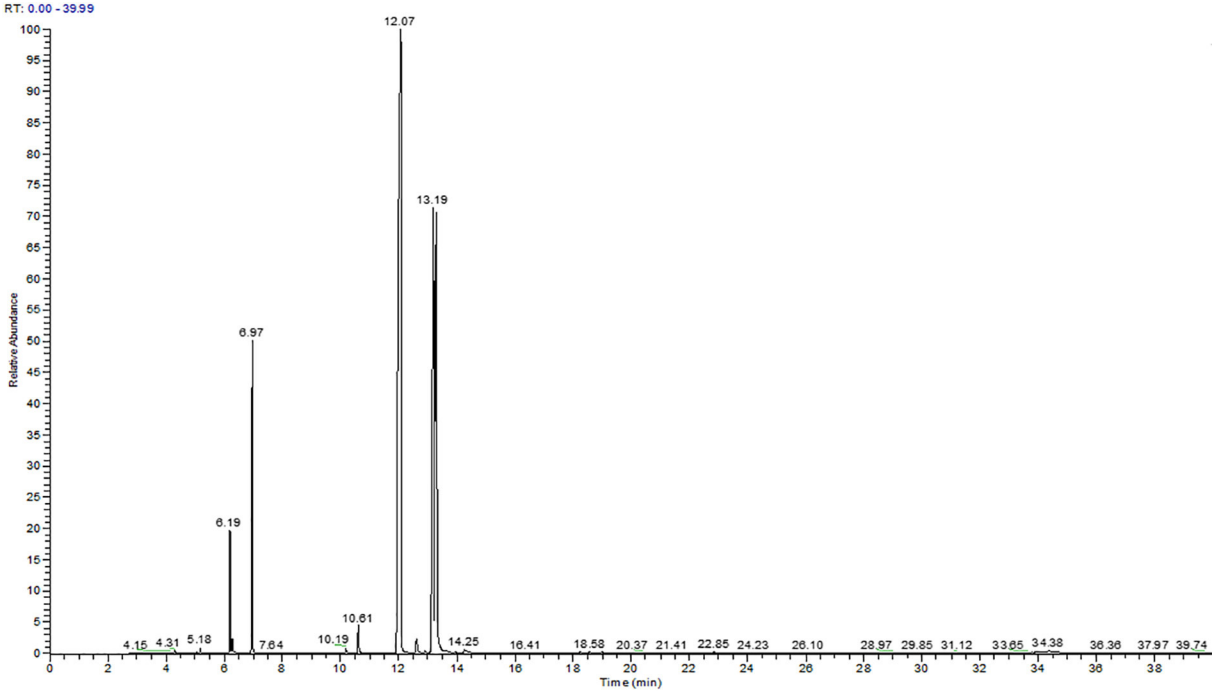


Figure 2. The GC-MS chromatograms of Bunium persicum essential oil.

Interlobular foci of hemorrhage and edema (5.3%) were observed in more than 60% of cases (Table 4, Figure 3).

Biochemical Analysis

MDA levels in the sham, *pre-IP+I/R*, *post-G+I/R*, and *post-IP+I/R* groups were significantly higher than those in the positive control group. In addition, MDA levels in the *post-IP+I/R* group were higher following treatment with BC essential oil than those in the negative control group (Figure 3).

SOD activity was assessed in rat plasma. Pretreatment with BC essential oil before surgery in the *pre-G+I/R* group resulted in significantly higher SOD activity than in the *pre-IP+I/R* group, as shown in Figure 3.

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Table 4. Impact of treatment with black cumin (BC) essential oil (0.1 ml/kg) on pancreatic morphological symptoms of pancreatic injury in I/R-induced acute pancreatitis. Pre-G+I/R and pre-IP+I/R*:* rats were given BC daily via oral gavage or IP, for 7 days before operation, respectively. Post G+I/R and post IP+I/R*:* rats were given BC through oral gavage or IP, for 1 day after the operation. Numbers show the predominant histological grading in each group.

Groups	Edema (0-3)	Inflammation (0-3)	Necrosis (0-3)	Hemorrhage (0-3)
Cont+	0	0	0	0
Cont-	0-1	0-1	0	0
Sham	1-2	1	2-3	1-2
pre-G+I/R	1-2	1-2	1	2
pre-IP+I/R	1	2	0-1	1
post G+I/R	1	1-2	0	1
post IP+I/R	2-3	2-3	1	1-2

Gene Expression (RT-qPCR)

Molecular analysis revealed no statistically significant differences in the expression levels of *Bcl2* and *p53* genes among the experimental and control groups (P>0.05) (Figure 4).

Discussion

The present study demonstrates that the prophylactic administration of *Bunium persicum* essential oil (BEO) provides significant protection against ischemia/reperfusion (I/R)-induced acute pancreatitis in a rat model. Our findings suggest that this protective effect is mediated by the attenuation of oxidative stress and the preservation of pancreatic tissue architecture, potentially through the modulation of systemic redox homeostasis.

Our *in vivo* histological findings indicate that pretreating rats with BC essential oil for 7 days before surgery reduces the number of necrotic foci, interlobular hemorrhages, and areas of edema, which are characteristic manifestations

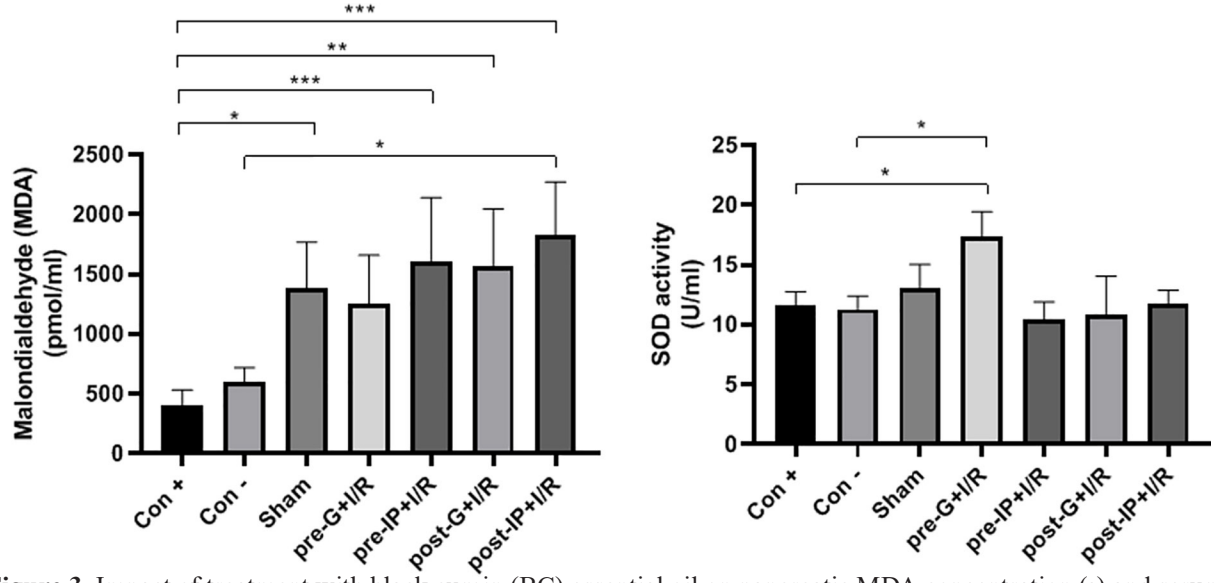


Figure 3. Impact of treatment with black cumin (BC) essential oil on pancreatic MDA concentration (a) and serum activity of SOD (b) of ischemia/reperfusion (I/R)-induced acute pancreatitis. Essential oil was given at a dose of 0.1 ml/kg in the positive control without pancreatitis, negative control with IP saline injection, sham rats with I/R-induced pancreatitis, and pre-and post-treated rats with I/R-induced pancreatitis. Pre-and post-treatment with gavage or IP injection was performed 7 days before *(pre-G+I/R and pre-IP+I/R)* and 1 day after *(post G+I/R and post IP+I/R)* surgery, respectively.

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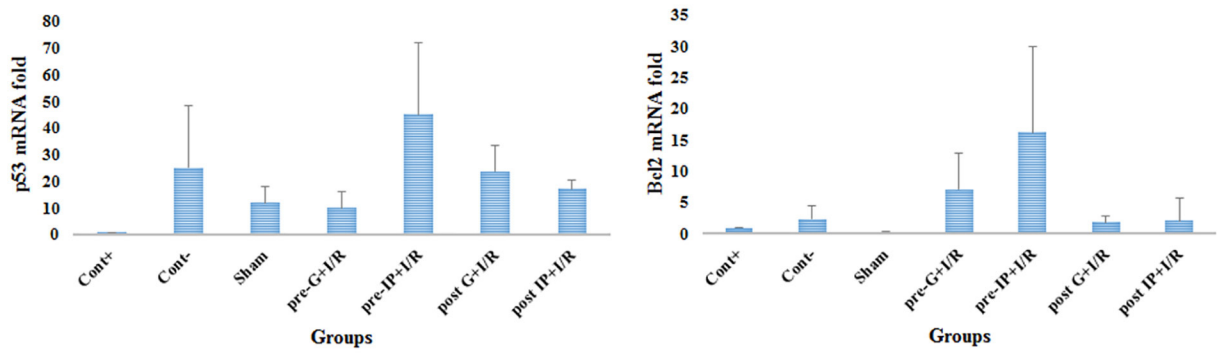


Figure 4. The mRNA fold expression (mean ± SEM) of Bcl2 and p53 genes relative to β-actin in controls, sham, and experimental (0.1 ml/kg of black cumin essential oil 7 days before surgery through gavage or IP injection as pre-G+I/R and pre-IP+I/R groups, respectively or 1 day after surgery via gavage or IP injection as post G+I/R and post IP+I/R, separately). The Bcl2 and p53 gene expression had no significant difference between groups ($P > 0.05$).

of pancreatic tissue damage during I/R-induced acute pancreatitis. Organ blood flow supplies tissues with sufficient oxygen and nutrients while facilitating the removal of carbon dioxide and metabolic waste products. Accordingly, pancreatic ischemia followed by reperfusion may be a primary cause of acute pancreatitis (16). Furthermore, early disruption of pancreatic circulation has been observed in acute pancreatitis induced primarily by nonvascular mechanisms (17), and the severity of acute pancreatitis has been correlated with the degree of impairment in pancreatic circulation (18). The essential role of adequate blood flow in organ regeneration has also been demonstrated in other parts of the digestive system (19). Black cumin is a traditional medicinal plant with various therapeutic properties, including antibacterial and antioxidant effects (13). Similar protective and therapeutic effects on the pancreas have been reported following the administration of acenocoumarol, warfarin, and ghrelin. These effects were accompanied by improvements in pancreatic blood flow in rats with I/R-induced acute pancreatitis and were associated with accelerated recovery (8). Thus, improved pancreatic blood flow may contribute to the reduction of several indicators of tissue damage during BC treatment for I/R-induced acute pancreatitis.

The potential anti-apoptotic activity of BC essential oil was investigated in rats with *in vivo* I/R-induced acute pancreatitis. Our results showed that the highest, although statistically nonsignificant, levels of *Bcl-2* and *p53* gene expression occurred in the *pre-IP+I/R* group. The sham-operated animals exhibited lower *Bcl-2* expression, whereas *p53* expression was lowest in the positive control group. An overall trend observed in the present study was a nonsignificant decrease in the expression of the anti-apoptotic gene *Bcl-2* following the development of I/R-induced acute pancreatitis. This change was consistent with the observed pathological and biochemical findings. This finding is consistent with a previous report demonstrating the apoptotic activity of BC ethanol extracts and suggesting their potential as a source of novel apoptotic agents for human leukemia cell lines (20). A study investigating the effects of BC on MDA levels and *p53* expression in pancreatic tissues of alloxan-induced rats reported that administration of BC seed oil (BCSO) at a dose of 68 mg/kg body weight per day decreased blood MDA and glucose levels while increasing *p53* expression (21). I/R-induced pancreatitis has been reported to activate the *p53* gene, followed by oxidative stress and pancreatic cell injury. BC contains thymoquinone and unsaturated fatty

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acids, which possess antioxidant properties. Thymoquinone has been proposed to inhibit pancreatic damage and prevent hyperglycemia through mechanisms involving *p53*. The *p53* protein plays important roles in regulating responses to environmental stressors and is activated by DNA damage induced by ROS generated during inflammation (22).

GC-MS profiling of *Bunium persicum* essential oil (BEO) identified cuminaldehyde (47.61%) and the terpinen-7-al isomers as its predominant constituents. This phytochemical profile is consistent with emerging evidence that plant-derived volatile compounds may mitigate ischemia/reperfusion (I/R) injury by modulating multiple molecular pathways, including oxidative stress and inflammatory cascades (2). Given that pancreatic I/R injury is characterized by substantial mitochondrial dysfunction and subsequent apoptotic signaling, the presence of these specific monoterpenoids may be particularly relevant (23).

Cuminaldehyde, in particular, has emerged as a potential therapeutic candidate for inflammatory and oxidative conditions because of its ability to neutralize reactive oxygen species (ROS) and suppress pro-inflammatory signaling (14). In the present study, the observed attenuation of I/R-induced damage may have resulted from synergistic interactions between cuminaldehyde and terpinen-7-al derivatives, which may collectively stabilize mitochondrial membranes and modulate apoptosis-related markers, such as *p53* and *Bcl-2*. Such multicomponent synergy is characteristic of essential oils and may provide a broader therapeutic spectrum than that offered by isolated compounds (24, 25).

Furthermore, the biological effects of BEO depend on the bioavailability and tissue-specific delivery of its volatile constituents, which determine their capacity to modulate systemic and local immune responses (26). The established pharmacological potential of *B. persicum* in various experimental models (15, 27) further supports its value as a source of potent bioactive compounds. Collectively, our findings suggest that BEO exerts cytoprotective effects through coordinated antioxidant, anti-inflammatory, and anti-apoptotic mechanisms, primarily driven by its high cuminaldehyde content.

The effects of ischemia on the human pancreas and the associated activation of ROS have been demonstrated previously (28). Under physiological conditions, oxygen utilization during normal metabolism generates low levels of ROS, which are safely neutralized by antioxidant defense mechanisms involving enzymes such as SOD (29). Physiological levels of ROS function as signaling molecules that regulate transcription and help protect against invading microorganisms. When ROS production exceeds the capacity of the antioxidant defense system, cellular proteins, DNA, and lipids become susceptible to damage. Peroxidation of cellular lipids (lipid peroxidation) is an early stage of ROS-mediated cellular injury, potentially leading to the loss of cellular integrity and damage to intracellular membranes (30). Such damage can lead to the intracellular leakage of proteases or the influx of calcium ions, ultimately resulting in necrosis (31). The present study provides further evidence of an association between the development of I/R-induced acute pancreatitis and ROS production. I/R was associated with an increase in the pancreatic concentration of MDA, a marker of oxidative lipid peroxidation. This finding is consistent with previous research indicating that acute oxidative stress plays an important role in the pathogenesis of acute pancreatitis (32).

In the present study, pretreatment with BC by oral gavage for 7 days before surgery did not significantly increase pancreatic oxidative stress and was associated with improved antioxidant defenses in rats with I/R-induced

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acute pancreatitis relative to the positive control group. Consistent with these findings, Dadkhah reported that BC oils may protect against dimethylhydrazine-induced premalignant lesions in the colon (33) and oxidative injury in kidney tissue during advanced stages of sepsis (34). The antioxidant properties of BC extracts and essential oil indicate their strong free radical-scavenging capacity and potential antioxidant effects (35). The *in vitro* antioxidant and antibacterial properties of BC oil have also been reported (36). The high antioxidant activity of BC has been attributed to linalool, carvone, anethole, estragole, limonene, monoterpene alcohols, carvacrol, flavonoids, and other polyphenolic compounds (37). Daily consumption of BC has been recommended as a source of antioxidants, reportedly without adverse effects, suggesting that it could serve as a natural alternative to synthetic antioxidants (38).

Thus, the beneficial effects of BC against I/R-induced pancreatic injury may be attributable to bioactive compounds with free radical-scavenging properties. The present study provides evidence that the antioxidant effects of BC may contribute to the neutralization of ROS. A notable finding was the higher SOD activity observed following oral administration than following intraperitoneal (IP) injection. This difference may be attributable to route-dependent variations in the tissue uptake of BC constituents. It is possible that BC-derived compounds administered intraperitoneally were poorly taken up by tissues and rapidly excreted, whereas oral administration may have facilitated their absorption. Nevertheless, these proposed mechanisms require further investigation. Overall, the findings support a possible association between the attenuation of pancreatitis and the antioxidant effects of black cumin essential oil. Further studies involving larger sample sizes and additional laboratory analyses are needed to evaluate the efficacy and underlying mechanisms of action of *Bunium persicum* essential oil.

Conclusion

Ischemia/reperfusion-induced pancreatic injury was associated with a nonsignificant downward trend in the expression of *Bcl-2*, an anti-apoptotic gene, consistent with the observed pathological and biochemical changes. Pretreatment with BC by oral gavage for 7 days before surgery ameliorated the morphological signs of pancreatic damage. These effects were accompanied by a nonsignificant change in MDA levels and increased SOD activity. Thus, the present findings provide further evidence of a possible association between the attenuation of pancreatitis and the antioxidant properties of this medicinal plant.

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Conflict of Interest

The authors declare no conflict of interest.

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Ethical Consideration

Forty-nine adult male *Wistar* rats weighing 250 to 300 g were obtained from the animal facility of Fasa University of Medical Sciences. The animals were maintained under standard housing conditions, and all experiments were conducted in accordance with relevant protocols and guidelines. The study was approved under ethical approval code 95066 (IR.FUMS.REC.1395.99).

Code of Ethics

All experimental procedures were performed

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in strict accordance with relevant institutional protocols and ethical guidelines. The study was approved by the Institutional Ethics Committee under approval code IR.FUMS.REC.1395.99.

Author's Contribution

ME.A. planned the study. M.N., A. M, A.T., and Z.M. did trials. Z.M. wrote the first draft. All authors contributed to the analysis of data and revision of the draft manuscript.

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