



OPEN Therapeutic effects of resveratrol and β -carotene on L-arginine-induced acute pancreatitis through oxidative stress and inflammatory pathways in rats

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Acute pancreatitis (AP) is a severe inflammatory condition affecting the pancreas, often leading to systemic inflammation and organ dysfunction. This study evaluated the effects of resveratrol (RES) and β -carotene (β C) on L-arginine-induced AP in rats. Forty-eight male Sprague Dawley rats were divided into six groups: Control (C), RES (20 mg/kg), β C (50 mg/kg), AP, AP + RES, and AP + β C. The AP model was induced with 250 mg/100 g L-arginine intraperitoneally twice daily with a 1-h interval. The AP group showed significantly elevated oxidative stress (MDA) and reduced GSH levels ($p < 0.001$). Immunohistochemical (IHC) staining with anti-insulin antibody revealed reduced β + langerhans islet size in the AP group. qPCR analysis indicated significant upregulation of inflammatory genes NF- κ B, TNF- α , and IL-1 β ($p < 0.001$), and apoptotic genes Bax and Caspase-3, with downregulation of Bcl-2 ($p < 0.001$). RES and β C treatments significantly reduced MDA levels and increased GSH levels ($p < 0.01$ for both) compared to the AP group. The AP + RES and AP + β C groups exhibited preserved β + Langerhans islet size ($p < 0.01$), suppressed NF- κ B, TNF- α , and IL-1 β expression, reduced Bax and Caspase-3 levels, and increased Bcl-2 levels ($p < 0.01$). Histopathological findings supported these results. RES and β C confer significant effects against L-arginine-induced acute pancreatitis by reducing oxidative stress, preserving pancreatic islet integrity, suppressing inflammatory responses, and modulating apoptotic pathways. RES demonstrated a slightly superior efficacy in reducing inflammation and oxidative stress markers, suggesting it may be more effective in treating acute pancreatitis.

Keywords Acute pancreatitis, Resveratrol, β -carotene, Oxidative stress, Inflammation, Apoptosis, L-arginine

Acute pancreatitis (AP) is a severe inflammatory condition primarily affecting the pancreas, often leading to systemic inflammation and multiple organ dysfunction, necessitating frequent hospitalization¹. AP ranges from 13 to 45 cases per 100,000 persons per year. The annual incidence of AP has increased with an average annual percent change of 3.07%, showing significant geographical variation with highest rates reported in Western countries. AP is associated with significant morbidity and mortality, with hospitalization costs exceeding \$30,000 per person in the United States. While overall mortality is approximately 1%, this rate can increase dramatically to 30–40% among hospitalized patients with organ failure or pancreatic necrosis^{2,3}. The etiology of AP includes various factors such as excessive alcohol consumption, gallstones, hyperlipidemia, physical trauma, viral infections, sepsis, shock, and adverse drug reactions⁴.

During the early stages of AP, the excessive production of reactive oxygen species (ROS) by pancreatic acinar cells and activated immune cells significantly exacerbates pancreatic tissue damage⁵. This oxidative stress is

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reflected in elevated levels of biomarkers such as malondialdehyde (MDA) and decreased levels of glutathione (GSH), which are critical indicators of lipid peroxidation and antioxidant capacity, respectively⁶.

Damaged pancreatic cells release damage-associated molecular patterns like high-mobility group box 1 (HMGB1), which acts as a potent pro-inflammatory cytokine. HMGB1 interacts with receptors such as toll-like receptor 4 (TLR4), triggering the activation of nuclear factor kappa B (NF- κ B). NF- κ B is a key transcription factor that, upon activation, translocates to the nucleus and stimulates the expression of various inflammatory cytokines, including tumor necrosis factor- α (TNF- α) and interleukin-1 beta (IL-1 β)^{7,8}. The upregulation of these cytokines leads to a cascade of inflammatory responses, contributing to the severity and progression of AP⁷. TNF- α and IL-1 β further amplify the inflammatory response by recruiting additional immune cells to the site of injury, exacerbating tissue damage and promoting a cycle of ongoing inflammation⁹.

Addressing the pathophysiological mechanisms of AP requires therapeutic strategies that focus on both suppressing the extensive inflammatory response and enhancing cytoprotective signaling⁶. Resveratrol (RES), a natural polyphenolic compound with 3,5,4'-trihydroxystilbene structure, exhibits potent antioxidant properties through direct free radical scavenging and enhancement of endogenous antioxidant systems¹⁰. β -carotene (β C), a lipid-soluble tetraterpene precursor of vitamin A, demonstrates significant membrane-protective effects through its conjugated double bond system that effectively neutralizes reactive oxygen species¹¹. RES has demonstrated significant therapeutic efficacy in experimental pancreatic injury models through NF- κ B pathway modulation¹², while β C has shown protective effects against oxidative damage in pancreatic tissue and has been successfully used in post-ERCP pancreatitis prevention¹³. Despite these established therapeutic properties, the potential effects of RES and β C in the context of L-arginine-induced AP remain underexplored, particularly regarding their influence on inflammatory pathways and oxidative stress markers.

Therefore, the aim of our study was to evaluate the effects of RES and β C on L-arginine-induced AP in rats. We focused on several key parameters: levels of oxidative stress markers (MDA and GSH) in pancreatic tissues, immunohistochemical staining with anti-insulin antibody to assess β + Langerhans islets, relative mRNA transcript levels of inflammatory genes (NF- κ B, TNF- α , and IL-1 β), and relative mRNA transcript levels of apoptotic genes (Bax and Caspase-3) and the anti-apoptotic gene (Bcl-2) in pancreatic tissues. Through these analyses, we aimed to elucidate the mechanisms by which RES and β C confer their effects against AP.

Materials and methods

Animals

A total of 48 male Sprague Dawley rats, aged 2 months and weighing 250 g, were obtained from Atatürk University Medical Experimental Application and Research Center. The rats were acclimatized to the laboratory conditions for one day before the commencement of the experiment and housed under controlled temperature and light conditions, with ad libitum access to food and water. The study strictly adhered to the ARRIVE guidelines and was conducted following the national regulations for the ethical use and care of laboratory animals. This study protocol was approved by Atatürk University Animal Experiments Local Ethics Committee (Decision No: 2023/13). All procedures performed in studies involving animals were in accordance with the ethical standards of the institution.

Drugs and chemical reagents

Chemicals utilized in this study included resveratrol, β -carotene, L-arginine were purchased from Sigma-Aldrich (USA). All chemicals utilized in the experiment were sourced from the highest-quality commercially available options.

Experimental design

The rats were randomly divided into six groups (n = 8 per group) as follows:

Control (C) group Rats in this group received 1 ml isotonic intraperitoneally (i.p.).

Resveratrol (RES) group Healthy rats in this group were given 20 mg/kg RES by gavage for 7 days¹⁴, with no changes to standard feeding conditions.

β -carotene (β C) group Rats in this group were administered 50 mg/kg β C by gavage for 7 days¹⁵.

Acute Pancreatitis (AP) group Experimental pancreatitis was induced in rats by administering 250 mg/100 g L-arginine i.p. twice daily with a 1-h interval¹⁶, with no changes to standard feeding conditions.

Acute Pancreatitis + Resveratrol (AP + RES) group One hour after the second L-arginine injection, rats in this group received 20 mg/kg RES by gavage at the same dose and time daily for 7 days. No changes to standard feeding conditions were made.

Acute Pancreatitis + β -carotene (AP + β C) group One hour after the second L-arginine injection, rats in this group received 50 mg/kg β C by gavage at the same dose and time daily for 7 days. No changes to standard feeding conditions were made.

Blood and tissue sampling

The duration of the experiment was seven days. At the end of the experimental period, rats were fasted overnight and deeply anesthetized using a combination of intraperitoneal thiopental sodium (20 mg/kg) and inhalation anesthesia with 5% sevoflurane. The depth of anesthesia was confirmed by the absence of pedal withdrawal reflexes. Blood samples were collected via cardiac puncture under anesthesia. The animals were then euthanized by administering a high dose of thiopental sodium (50 mg/kg). Pancreatic tissues were immediately excised and processed under sterile conditions. Tissue samples were either snap-frozen in liquid nitrogen and stored at -80°C for biochemical analysis or fixed in 10% neutral buffered formalin for histopathological and immunohistochemical examination.

Histopathological methods

The tissues were processed through routine alcohol-xylol series and embedded in paraffin blocks. Sections of 5 μm thickness were taken on poly-L-lysine-coated slides, stained with hematoxylin–eosin, and examined for degenerative and necrotic changes in the endocrine and exocrine pancreas in 10 randomly selected areas¹⁷. The histopathological changes were scored based on the criteria presented in Table 1.

Evaluation of oxidative stress biomarkers

Pancreatic tissues were powdered using a homogenizer (Tissue Lyser II, Qiagen, Netherlands) under liquid nitrogen. For MDA level analysis, the tissues were diluted at a 1:10 ratio with 1.15% phosphate buffer and homogenized with stainless steel beads using the same device. The homogenates were centrifuged at 3500 RPM for 15 min, and the supernatants were used to determine MDA levels, an indicator of lipid peroxidation, following the method of Ohkawa et al.¹⁸. For GSH level analysis, the tissues were also diluted at a 1:10 ratio and homogenized, followed by centrifugation at 10,000 RPM for 20 min. The supernatants were analyzed for GSH levels using the method based on the reduction of Ellman's reagent [5,5'-dithiobis (2-nitrobenzoic acid)]¹⁹.

Immunohistochemical methods

The tissue sections of 5 μm thickness were deparaffinized, rehydrated through xylol and alcohol series, and washed with PBS. Endogenous peroxidase activity was blocked by incubating the sections in 3% H_2O_2 for 10 min. Antigen retrieval was performed using an antigen retrieval solution in a microwave at 500 watts for 2 \times 5 min. After washing with PBS, the tissues were incubated overnight at +4 $^\circ\text{C}$ with anti-Insulin primary antibody (Sigma, Cat. no. I2018) at a 1/500 dilution. The secondary antibody, Large Volume Detection System: anti-Polyvalent, HRP (ThermoFischer, Cat. no: TP-125-HL), was applied according to the manufacturer's instructions. DAB (3,3'-Diaminobenzidine) was used as the chromogen. After counterstaining with Mayer's hematoxylin, the sections were mounted with entellan and examined under a light microscope. The diameter and area of 10 randomly selected Langerhans islets with immunopositivity (diameter and area) were measured using Leica LAS EZ software²⁰.

Quantitative real-time polymerase chain reaction (qRT-PCR.) analyses

100 mg of pancreatic tissues were weighed and 1 mL of QIAzol Lysis Reagent (79,306; Qiagen) was added. Then, the tissues were homogenized for 1 min in a homogenizer (Tissue Lyser II, Qiagen, Netherlands) with the help of stainless steel balls, and then the homogenates were incubated at room temperature for 5 min. After the incubation period was completed, 200 μL of chloroform was added to the samples and incubated for 3 min after vortexing. The tubes were then centrifuged at 12,000 g for 15 min. The upper transparent phase was transferred to new sterile tubes and 500 μL of isopropanol was added. Samples incubated for 10 min were centrifuged at 12,000 g for 10 min. Then, isopropanol was removed and the pellet was washed with 1 mL of 75% ethanol. Centrifugation was performed at 7500 g for 5 min to remove ethanol. Ethanol was removed from the tubes and the pellet was dissolved with DNase/RNase free water. The concentration and RNA integrity of the obtained total RNA solution were measured on the NanoDrop (BIO-TEK INSTRUMENTS EPOCH, USA) device. The quality of RNAs was determined according to the 260/280 ratio. Accordingly, RNA samples with a 260/280 ratio of 2.0 were used in cDNA synthesis. cDNA synthesis was performed using the iScript cDNA Synthesis Kit (Bio-Rad). All procedures were performed by strictly following the manufacturer's instructions. For this purpose, a mixture was prepared by adding 5 μL 5 \times iScript Reaction Mix, 1 μL iScript Reverse Transcriptase and 4 μL iScript Nuclease-free water to 10 μL total RNA. The mixture was then incubated at 25 $^\circ\text{C}$ for 5 min, 46 $^\circ\text{C}$ for 20 min and 95 $^\circ\text{C}$ for 1 min in the ROTOR-GENE Q (Qiagen, Germany) device to generate cDNAs. After the concentrations of cDNAs were measured on the NanoDrop (BIO-TEK INSTRUMENTS EPOCH, USA) device, the qRT-PCR stage was started. For quality control of cDNAs, the 260/280 ratio was taken as reference. cDNAs with a 260/280 ratio of 1.8 were used. The synthesized cDNA was then subjected to qRT-PCR using the primers listed in Table 2 and iTaq Universal SYBR Green Supermix (Bio-Rad). The reactions were performed in a Rotor-Gene Q (Qiagen) machine according to the manufacturer's instructions. Gene expression was normalized to β -Actin using the $2^{-\Delta\Delta\text{CT}}$ method²¹.

Statistical analysis

Histopathological and immunohistochemical data were analyzed using IBM SPSS, version 20.0 (IBM, USA; URL: <https://www.ibm.com/products/spss-statistics>). The differences between groups for histopathological data were analyzed using the non-parametric Kruskal–Wallis test, and the groups causing the differences were identified using the Mann–Whitney U test ($p < 0.05$). Immunohistochemical data were analyzed using one-way ANOVA followed by post hoc Tukey's test ($p < 0.001$).

Histological appearance	Degenerative changes
Normal	None (-)
< 30% Degenerative cells	Mild (+)
30–60% Degenerative cells	Moderate (++)
> 60% Degenerative cells	Severe (+++)

Table 1. Histopathological scoring.

Gene	Sequences (5'-3')	Length (bp)	Accession No
Bcl-2	F: GACTTTGCAGAGATGTCCAG	214	NM_016993.2
	R: TCAGGTAICTCAGTCATCCAC		
Caspase-3	F: ACTGGAATGTGAGCTCGCAA	270	NM_012922.2
	R: GCAGTAGTCGCCTCTGAAGA		
Bax	F: TTTCATCCAGGATCGAGCAG	154	NM_017059.2
	R: AATCATCCTCTGCAGCTCCA		
NF-κB	F: AGTCCCGCCCTTCTAAAAC	106	NM_001276711.1
	R: CAATGGCCTCTGTGTAGCCC		
IL-1β	F: ATGGCAACTGTCCCTGAACT	197	NM_031512.2
	R: AGTGACACTGCCTTCTGAA		
TNF-α	F: CTCGAGTGACAAGCCCGTAG	139	NM_012675.3
	R: ATCTGCTGGTACCACCAGTT		
β-Actin	F: CAGCCTTCCTTCTGGGTATG	360	NM_031144.3
	R: AGCTCAGTAACAGTCCGCCT		

Table 2. List of primer sequences.

Groups	Endocrine pancreas	Exocrine pancreas
Control (C)	0.16 ± 0.40 ^a	0.33 ± 0.51 ^a
Resveratrol (RES)	0.33 ± 0.51 ^a	0.16 ± 0.40 ^a
β-Carotene (βC)	0.16 ± 0.40 ^a	0.16 ± 0.40 ^a
Acute pancreatitis (AP)	2.83 ± 0.40 ^b	2.83 ± 0.40 ^b
Acute pancreatitis + Resveratrol (AP + RES)	2.66 ± 0.51 ^b	1.83 ± 0.40 ^c
Acute pancreatitis + β-Carotene (AP + βC)	1.33 ± 0.51 ^c	1.00 ± 0.00 ^d

Table 3. Comparison of endocrine and exocrine pancreas among the groups. ^{a,b,c,d}Different letters within the same column indicate significant differences ($p < 0.05$).

Results

Histopathological findings

Histopathological evaluation revealed statistically significant differences among the groups (Table 3, $p < 0.05$). The AP group exhibited the most severe degenerative changes, while treatments with AP + RES and AP + βC mitigated these changes to varying extents.

As shown in Fig. 1, histopathological examinations showed that the pancreas of rats in the C, RES, and βC groups had normal histological appearances. In other treatment groups, various degrees of pathological findings were observed in both endocrine and exocrine pancreas. Severe degenerative-necrotic cells were found in the Langerhans islets of the endocrine pancreas in the AP and AP + RES groups, whereas these changes were mild in the AP + βC group. Degenerative-necrotic changes were also observed in the acinar cells of the exocrine pancreas. These changes were severe in the AP group, moderate in the AP + RES group, and mild in the AP + βC group (Fig. 1).

Oxidative stress markers in pancreatic tissues

The MDA and GSH levels in pancreatic tissues are presented in Fig. 2. The results indicate that lipid peroxidation occurred in the pancreatic tissues following acute pancreatitis (AP), as evidenced by increased MDA levels ($p < 0.001$) and decreased GSH stores ($p < 0.001$). On the other hand, treatments with RES and βC significantly reduced MDA levels ($p < 0.01$ for both) and increased GSH levels ($p < 0.01$ for both) compared to the AP group.

Immunohistochemical findings

Immunohistochemical staining for anti-insulin revealed statistically significant differences in the diameter and area of β + Langerhans islets among the groups (Table 4, $p < 0.001$).

Immunohistochemical analysis showed that the diameter and area of β + Langerhans islets were normal in the C, RES, and βC groups. In other treatment groups, these measurements varied significantly. The AP group had the smallest diameter and area of β + Langerhans islets, while the AP + RES group had larger diameters and areas, and the AP + βC group had the largest diameters and areas (Fig. 3).

Relative mRNA transcript levels of inflammatory genes in pancreatic tissues

To determine the inflammatory status in pancreatic tissues, the relative mRNA transcript levels of NF-κB, TNF-α, and IL-1β genes were analyzed, and the results are shown in Fig. 4. The data indicate that these genes were upregulated in the pancreatic tissues following AP ($p < 0.001$ for all). However, treatments with RES and βC

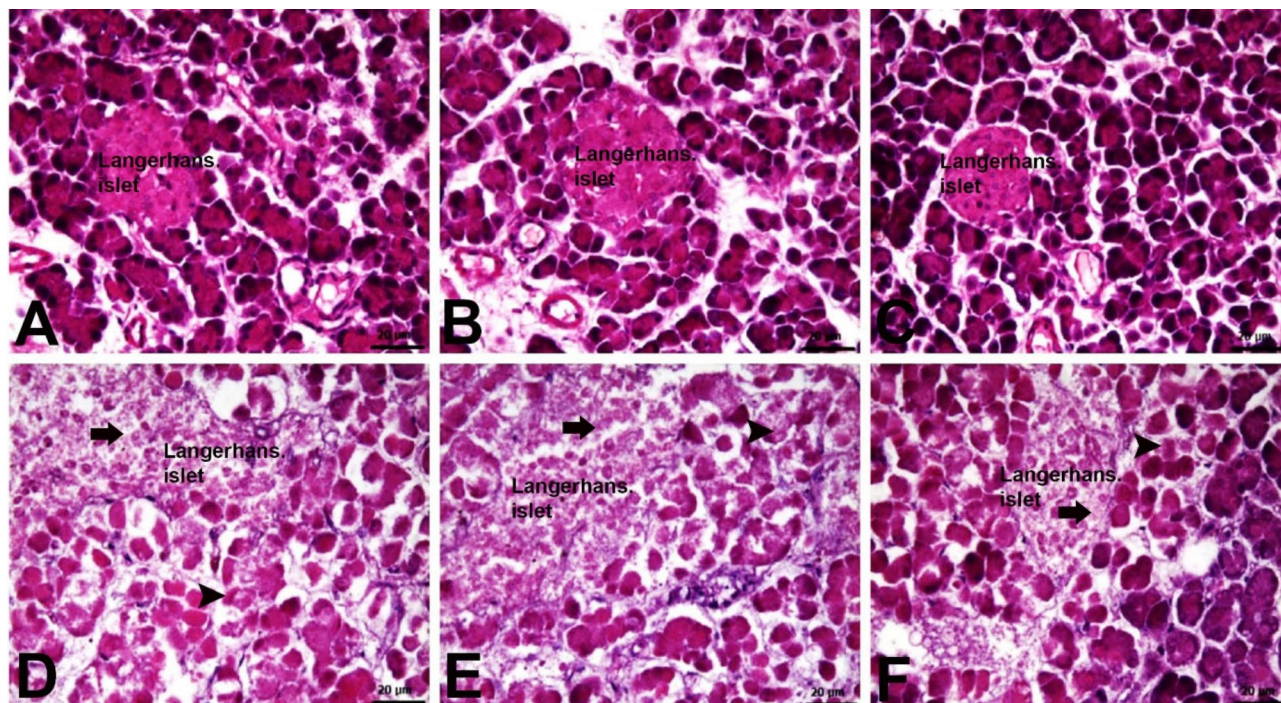


Fig. 1. Histopathological examination of the pancreas in different groups: (A) Control group, (B) RES group, (C) β C group. Normal histological appearance. (D) AP group. Severe degenerative-necrotic changes in the cells of Langerhans islets (arrow) and acinar cells (arrowhead). (E) AP+RES group. Severe degenerative-necrotic changes in the cells of Langerhans islets (arrow) and moderate changes in acinar cells (arrowhead). (F) AP+ β C group. Mild degenerative-necrotic changes in the cells of Langerhans islets (arrow) and acinar cells (arrowhead) (H&E staining, magnification $\times 40$).

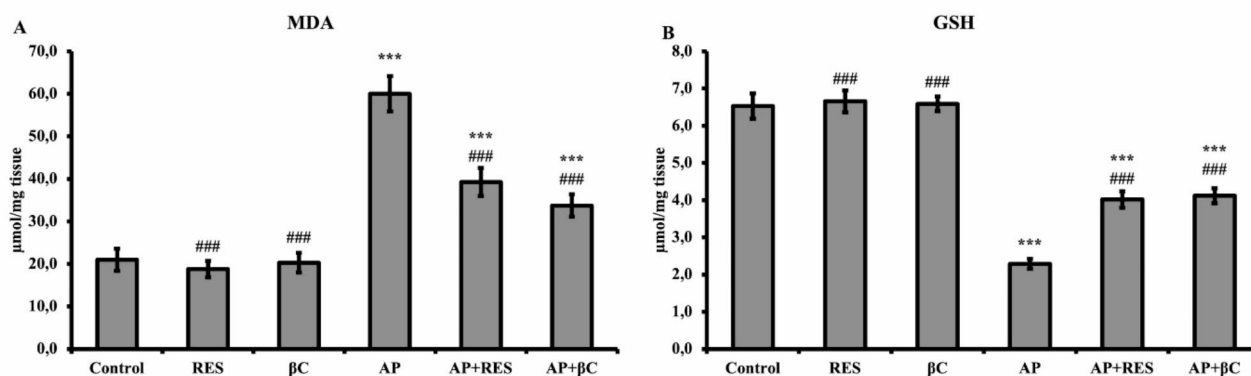


Fig. 2. Levels of oxidative stress markers MDA and GSH in pancreatic tissues. Values are expressed as mean \pm SD. Statistical significance: Control vs. others: *** $p < 0.001$; AP vs. others: ### $p < 0.001$.

Groups	Diameter of β + Langerhans islets (μm)	Area of β + Langerhans islets (mm^2)
Control (C)	55.16 \pm 1.47 ^a	2.371 \pm 0.25 ^a
Resveratrol (RES)	54.22 \pm 1.83 ^a	2.363 \pm 1.14 ^a
β -Carotene (β C)	53.34 \pm 2.63 ^a	2.359 \pm 0.83 ^a
Acute pancreatitis (AP)	11.68 \pm 1.28 ^b	0.078 \pm 0.18 ^b
Acute pancreatitis + Resveratrol (AP + RES)	23.75 \pm 0.96 ^c	0.314 \pm 1.06 ^c
Acute pancreatitis + β -Carotene (AP + β C)	34.81 \pm 2.17 ^d	1.040 \pm 1.32 ^d

Table 4. Comparison of the diameter and area of β + Langerhans islets among the groups. ^{a,b,c,d}Different letters within the same column indicate significant differences ($p < 0.001$).

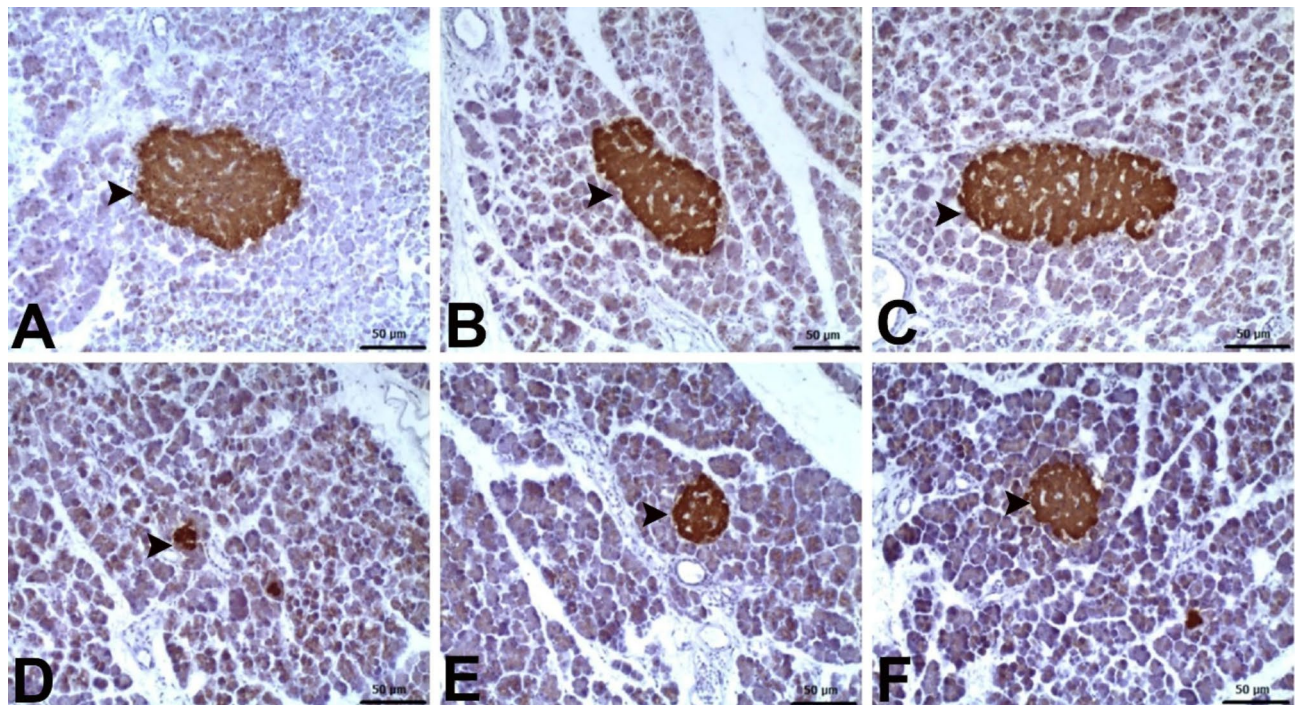


Fig. 3. Diameter and area of β + Langerhans islets (arrowhead) in different groups (immunohistochemical staining with anti-insulin antibody). (A) Control group, (B) RES group, (C) β C group. Normal histological appearance of β + Langerhans islets. (D) AP group. Group with the smallest β + Langerhans islets. (E) AP + RES group. Group with larger β + Langerhans islets. (F) AP + β C group. Group with the largest β + Langerhans islets (Immunohistochemistry, magnification $\times 20$).

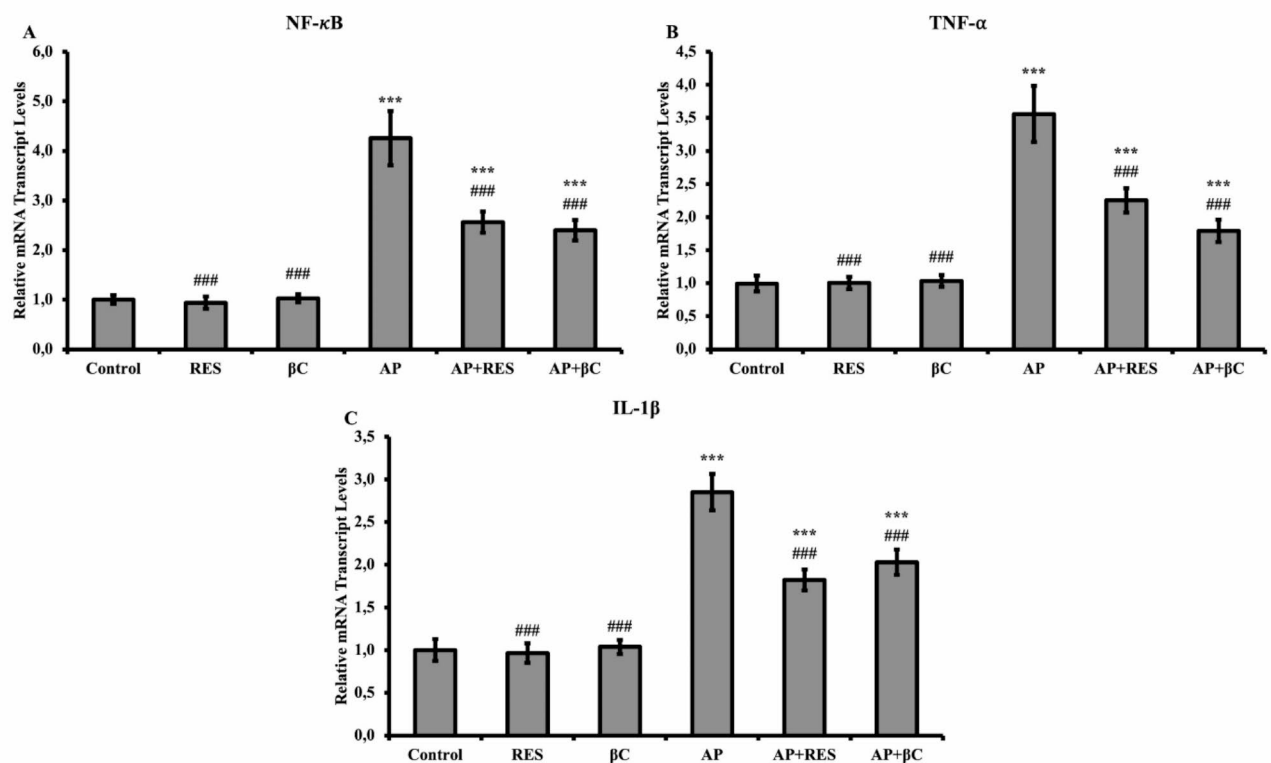


Fig. 4. Relative mRNA transcript levels of inflammatory genes NF- κ B, TNF- α , and IL-1 β in pancreatic tissues. Statistical significance: Control vs. others: *** $p < 0.001$; AP vs. others: ### $p < 0.001$.

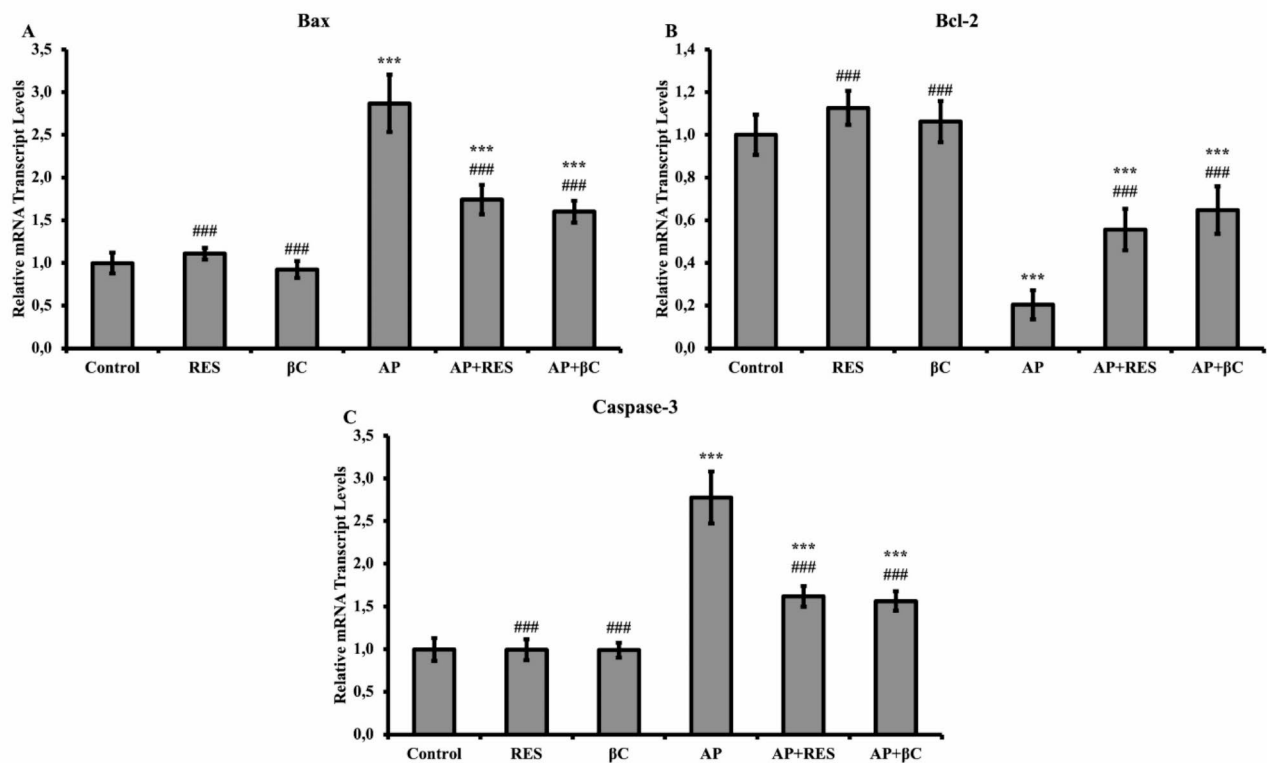


Fig. 5. Relative mRNA transcript levels of apoptotic genes Bax and Caspase-3, and anti-apoptotic gene Bcl-2 in pancreatic tissues. Statistical significance: Control vs. others: *** $p < 0.001$; AP vs. others: ### $p < 0.001$.

suppressed the expression of NF- κ B ($p < 0.01$ for both), TNF- α ($p < 0.01$ for both), and IL-1 β ($p < 0.01$ for both), demonstrating an anti-inflammatory effect.

Relative mRNA transcript levels of apoptotic and anti-apoptotic genes in pancreatic tissues

The relative mRNA transcript levels of the apoptotic genes Bax and Caspase-3 and the anti-apoptotic gene Bcl-2 in pancreatic tissues are shown in Fig. 5. The results indicate that AP induced an increase in the expression of Bax ($p < 0.001$) and Caspase-3 ($p < 0.001$) and a decrease in Bcl-2 levels ($p < 0.001$). Conversely, RES and β C treatments exhibited anti-apoptotic effects by downregulating Bax ($p < 0.01$ for both) and Caspase-3 ($p < 0.01$ for both) and upregulating Bcl-2 ($p < 0.01$ for both) compared to the AP group.

Discussion

In this study, we investigated the potential therapeutic properties of RES and β C against AP induced by L-arginine using a rat model. The utilization of L-arginine in the induction of AP serves as a straightforward and effective experimental model, enabling the examination of various histological and biochemical alterations that closely mimic AP in humans^{22,23}.

Histopathological assessment of the AP group revealed significant degenerative changes in both endocrine and exocrine pancreas, characterized by necrosis, edema, and inflammatory infiltration. Treatment with RES and β C effectively reduced these alterations, with β C showing superior efficacy in preserving pancreatic tissue integrity^{24–26}. Similarly, immunohistochemical analysis demonstrated that AP significantly reduced β + Langerhans islets size, while RES and β C treatments preserved islet structure and size, maintaining pancreatic endocrine function. These findings correspond with recent studies showing antioxidant-mediated preservation of β -cell viability under inflammatory conditions^{11,27}.

Oxidative stress is a critical factor in the pathogenesis of various diseases, including AP. The excessive formation of ROS during AP leads to lipid peroxidation, protein oxidation, and DNA damage, exacerbating cellular injury²⁸. The levels of MDA, a lipid peroxidation product, reflect the level of free radicals in tissues²⁹. Glutathione deficiency and the involvement of oxygen free radicals have been linked to acute pancreatitis in humans³⁰. In this study, we observed significantly elevated levels of MDA, a marker of lipid peroxidation, and decreased levels of GSH, a crucial antioxidant, in the AP group. These changes highlight the imbalance between ROS production and antioxidant defenses in AP and these findings are consistent with the literature^{31,32}. Studies have shown that treatments with RES and β C significantly reduced oxidative stress and restored antioxidants levels, suggesting that these compounds enhance the antioxidant defense system and mitigate oxidative stress. It has been shown that RES, with its potent antioxidant properties, could protect the intestinal tissue from I/R injury and could have a beneficial effect in inhibiting the oxidative damage in the liver induced by the chronic administration of ethano^{33,34}.

Inflammation is a hallmark of acute pancreatitis and is driven by the activation of various inflammatory pathways³⁵. NF- κ B is a central molecule that regulates the expression of many pro-inflammatory cytokines and mediators. Its activation leads to the recruitment of immune cells, exacerbating tissue injury and inflammation³⁶. Previous studies have confirmed that NF- κ B activation during AP is capable of producing numerous types of cytokines such as TNF- α , IL-1 β , IL-2, IL-6 and IL-18, and various chemokines such as IL-8^{37–39}. In this study, we found that, the relative mRNA transcript levels of NF- κ B, TNF- α , and IL-1 β were significantly upregulated in the L-Arginine induced AP group, reflecting an intense inflammatory response. Treatments with RES and β C significantly suppressed the expression of NF- κ B, TNF- α , and IL-1 β , demonstrating their potent anti-inflammatory effects. These findings are consistent with previous research showing the anti-inflammatory properties of RES. For instance, RES has been shown to decrease serum levels of TNF- α and IL-6 in severe acute pancreatitis (SAP) modeled rats, reduce mortality, and suppress the production, activation, and release of inflammatory mediators⁴⁰. Additionally, data have shown that NF- κ B levels in SAP rats increased after modeling, while RES inhibited NF- κ B activity and decreased the expression of TNF- α and IL-8, indicating that RES modulates cytokine expression by inhibiting NF- κ B and the inflammatory response⁴¹. Furthermore, studies have demonstrated that RES effectively reduces NF- κ B activation in inflammatory conditions, upregulating anti-inflammatory cytokines like IL-10 while suppressing pro-inflammatory mediators^{42,43}. Similarly, β C exhibits significant anti-inflammatory properties by modulating the TLR4 signaling pathway, enhancing barrier function, and reducing inflammatory agents^{44,45}. These findings align with our results showing that both compounds effectively suppress NF- κ B activation and downregulate pro-inflammatory cytokines, thereby alleviating pancreatic inflammation.

Apoptosis, or programmed cell death, is another critical aspect of acute pancreatitis⁴⁶. In current study, the imbalance between pro-apoptotic and anti-apoptotic factors determines the fate of pancreatic cells during L-arginine induced AP. The relative mRNA transcript levels of the pro-apoptotic genes Bax and Caspase-3 were significantly increased in the AP group, while the anti-apoptotic gene Bcl-2 was decreased, indicating enhanced apoptotic activity. Treatment with RES and β C exhibited anti-apoptotic effects by downregulating Bax and Caspase-3 and upregulating Bcl-2 levels. This suggests that RES and β C can modulate apoptotic pathways to protect pancreatic cells from programmed cell death, thus preserving pancreatic function. These findings are consistent with previous studies demonstrating the anti-apoptotic properties of antioxidants in various models of oxidative stress and inflammation^{9,11,31,44,47,48}. The interplay between oxidative stress, inflammation, and apoptosis in the context of acute pancreatitis creates a vicious cycle that exacerbates pancreatic injury. Concurrently, the imbalance between pro-apoptotic and anti-apoptotic signals results in increased apoptosis of pancreatic cells, further compromising pancreatic function^{49,50}.

Conclusion

RES and β C demonstrate therapeutic effects against L-arginine-induced acute pancreatitis through three key mechanisms: inhibition of the NF- κ B inflammatory pathway, reduction of oxidative stress, and modulation of apoptotic pathways. These effects are evidenced by decreased inflammatory markers (NF- κ B, TNF- α , IL-1 β), improved oxidative status (reduced MDA, increased GSH), and regulated apoptotic gene expression (decreased Bax/Caspase-3, increased Bcl-2). Consequently, RES and β C hold potential as therapeutic agents for the treatment of acute pancreatitis induced by L-arginine.

Data availability

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

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References

1. Mederos, M. A., Reber, H. A. & Girgis, M. D. Acute pancreatitis: A review. *JAMA* **325**(4), 382–390 (2021).
2. Gagyı, E.-B. et al. Incidence of recurrent and chronic pancreatitis after acute pancreatitis: A systematic review and meta-analysis. *Ther. Adv. Gastroenterol.* **17**, 17562848241255304 (2024).
3. Iannuzzi, J. P. et al. Global incidence of acute pancreatitis is increasing over time: A systematic review and meta-analysis. *Gastroenterology* **162**(1), 122–134 (2022).
4. Szatmary, P. et al. Acute pancreatitis: Diagnosis and treatment. *Drugs* **82**(12), 1251–1276 (2022).
5. Pérez, S., Pereda, J., Sabater, L. & Sastre, J. Redox signaling in acute pancreatitis. *Redox Biol.* **5**, 1–14 (2015).
6. Ali, B. M. et al. Pinocembrin's protective effect against acute pancreatitis in a rat model: The correlation between TLR4/NF- κ B/NLRP3 and miR-34a-5p/SIRT1/Nrf2/HO-1 pathways. *Biomed. Pharmacother.* **176**, 116854 (2024).
7. Li, L. et al. Toll-like receptor 2 deficiency alleviates acute pancreatitis by inactivating the NF- κ B/NLRP3 pathway. *Int. Immunopharmacol.* **121**, 110547 (2023).
8. Ren, W., Zhao, L., Sun, Y., Wang, X. & Shi, X. HMGB1 and Toll-like receptors: Potential therapeutic targets in autoimmune diseases. *Mol. Med.* **29**(1), 117 (2023).
9. Üstündağ, H., Demir, Ö., Huyut, M. T. & Yüce, N. Investigating the individual and combined effects of coenzyme Q10 and vitamin C on CLP-induced cardiac injury in rats. *Sci. Rep.* **14**(1), 3098 (2024).
10. Roshani, M. et al. Applications of resveratrol in the treatment of gastrointestinal cancer. *Biomed. Pharmacother.* **153**, 113274 (2022).
11. Sandoval, C. et al. β -carotene supplementation improves pancreas function during moderate ethanol consumption: Initial characterization from a morphological overview. *Int. J. Mol. Sci.* **25**(2), 1219 (2024).
12. Zhang, X. et al. Resveratrol pre-treatment alleviated caerulein-induced acute pancreatitis in high-fat diet-feeding mice via suppressing the NF- κ B proinflammatory signaling and improving the gut microbiota. *BMC Complement Med. Ther.* **22**(1), 189 (2022).
13. Lavy, A. et al. Natural β -carotene for the prevention of post-ERCP pancreatitis. *Pancreas* **29**(2), e45–e50 (2004).

14. Sarubbo, F. et al. Resveratrol improves episodic-like memory and motor coordination through modulating neuroinflammation in old rats. *J. Funct. Foods* **104**, 105533 (2023).
15. Giap, L. K., Varatharajan, R. & Muthuraman, A. Therapeutic investigations of palm oil induced beta-carotene in diabetic vascular dementia in rat. *Res. J. Pharm. Technol.* **16**(2), 566–572 (2023).
16. Mostafa, R. E., Abdelrahmen, S. S. & Saleh, D. O. L-Arginine-induced acute pancreatitis and its associated lung injury in rats: Down-regulation of TLR-4/MAPK-p38/JNK signaling pathway via Ginkgo biloba extract EGb 761. *Iran. J. Basic Med. Sci.* **27**(8), 959 (2024).
17. Gezer, A. et al. Effect of vitamin D3 and a stinging nettle extract on the gastric tissue of rats administered with trinitrobenzene sulfonic acid. *Vet. Med.* **69**(3), 84 (2024).
18. Ohkawa, H., Ohishi, N. & Yagi, K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal. Biochem.* **95**(2), 351–358 (1979).
19. Sedlak, J. & Lindsay, R. H. Estimation of total, protein-bound, and nonprotein sulfhydryl groups in tissue with Ellman's reagent. *Anal. Biochem.* **25**, 192–205 (1968).
20. Gezer, A., Laloglu, A. & Bölükbaş, M. K. Protective effects of alpha lipoic acid against ionizing radiation-Induced hepatotoxicity in rats. *Eurasian J. Med.* **55**(2), 104 (2023).
21. Zhao, F., Maren, N. A., Kosentka, P. Z., et al. An optimized protocol for stepwise optimization of real-time RT-PCR analysis. *Hortic. Res.* **8** (2018).
22. Siriviriyakul, P. et al. Genistein attenuated oxidative stress, inflammation, and apoptosis in L-arginine induced acute pancreatitis in mice. *BMC Complement Med. Ther.* **22**(1), 208 (2022).
23. Yang, X. et al. Experimental acute pancreatitis models: history, current status, and role in translational research. *Front. Physiol.* **11**, 614591 (2020).
24. Glaubitz, J. et al. Immune response mechanisms in acute and chronic pancreatitis: Strategies for therapeutic intervention. *Front. Immunol.* **14**, 1279539 (2023).
25. Mitra, S., Bhattacharyya, S., Ray, S. et al. Resveratrol alleviates cadmium-induced damage and overexpression of epidermal growth factor receptor and its downstream signaling proteins in the reproductive system of male Swiss albino mice. *J. Environ. Pathol. Toxicol. Oncol.* **35** (1) (2016).
26. Yang, X. et al. Experimental acute pancreatitis models: History, current status, and role in translational research. *Front. Physiol.* **11**, 614591 (2020).
27. Nimbalkar, V., Joshi, U., Shinde, S. & Pawar, G. In-vivo and in-vitro evaluation of therapeutic potential of β -Carotene in diabetes. *J. Diabetes Metab. Disord.* **20**, 1621–1630 (2021).
28. Cai, Y., Yang, F. & Huang, X. Oxidative stress and acute pancreatitis. *Biomed. Rep.* **21**(2), 1–7 (2024).
29. Mas-Bargues, C. et al. Lipid peroxidation as measured by chromatographic determination of malondialdehyde. Human plasma reference values in health and disease. *Arch. Biochem. Biophys.* **709**, 108941 (2021).
30. Ma, Q., Zhang, M., Wang, Z., Ma, Z. & Sha, H. The beneficial effect of resveratrol on severe acute pancreatitis. *Ann. N. Y. Acad. Sci.* **1215**(1), 96–102 (2011).
31. Mirmalek, S. A. et al. Antioxidant and anti-inflammatory effects of coenzyme Q10 on L-arginine-induced acute pancreatitis in rat. *Oxid. Med. Cell Longev.* **2016**, 5818479 (2016).
32. Tang, Q.-q, Su, S.-y & Fang, M.-y. Zinc supplement modulates oxidative stress and antioxidant values in rats with severe acute pancreatitis. *Biol. Trace Elem. Res.* **159**, 320–324 (2014).
33. Kasdallah-Grissa, A. et al. Resveratrol, a red wine polyphenol, attenuates ethanol-induced oxidative stress in rat liver. *Life Sci.* **80**(11), 1033–1039 (2007).
34. Ozkan, O. V. et al. Resveratrol, a natural antioxidant, attenuates intestinal ischemia/reperfusion injury in rats. *Tohoku J. Exp. Med.* **218**(3), 251–258 (2009).
35. Peng, C., Li, Z. & Yu, X. The role of pancreatic infiltrating innate immune cells in acute pancreatitis. *Int. J. Med. Sci.* **18**(2), 534 (2021).
36. Jakkampudi, A. et al. NF- κ B in acute pancreatitis: Mechanisms and therapeutic potential. *Pancreatolgy* **16**(4), 477–488 (2016).
37. Virlos, I. et al. Calpain I inhibitor ameliorates the indices of disease severity in a murine model of cerulein-induced acute pancreatitis. *Intensiv. Care Med.* **30**, 1645–1651 (2004).
38. Viterbo, D. et al. Pancreatitis-associated protein 2 modulates inflammatory responses in macrophages. *J. Immunol.* **181**(3), 1948–1958 (2008).
39. Zhang, M.-S., Zhang, K.-J., Zhang, J. et al. Phospholipases A-II (PLA2-II) induces acute pancreatitis through activation of the transcription factor NF-kappaB. *Eur. Rev. Med. Pharmacol. Sci.* **18** (8) (2014).
40. Ma, Z.-H. et al. Effect of resveratrol on peritoneal macrophages in rats with severe acute pancreatitis. *Inflamm. Res.* **54**, 522–527 (2005).
41. Shigematsu, S. et al. Resveratrol, a red wine constituent polyphenol, prevents superoxide-dependent inflammatory responses induced by ischemia/reperfusion, platelet-activating factor, or oxidants. *Free Radic. Biol. Med.* **34**(7), 810–817 (2003).
42. Fuggetta, M. P. et al. Downregulation of proinflammatory cytokines in HTLV-1-infected T cells by Resveratrol. *J. Exp. Clin. Cancer Res.* **35**, 1–9 (2016).
43. Palacz-Wrobel, M. et al. Effect of apigenin, kaempferol and resveratrol on the gene expression and protein secretion of tumor necrosis factor alpha (TNF- α) and interleukin-10 (IL-10) in RAW-264.7 macrophages. *Biomed. Pharmacother.* **93**, 1205–1212 (2017).
44. Akkara, P. J. & Sabina, E. P. Pre-treatment with beta carotene gives protection against nephrotoxicity induced by bromobenzene via modulation of antioxidant system, pro-inflammatory cytokines and pro-apoptotic factors. *Appl. Biochem. Biotechnol.* **190**, 616–633 (2020).
45. Cheng, J., Balbuena, E., Miller, B. & Eroglu, A. The role of β -carotene in colonic inflammation and intestinal barrier integrity. *Front. Nutr.* **8**, 723480 (2021).
46. Al Mamun, A. et al. Pyroptosis in acute pancreatitis and its therapeutic regulation. *Apoptosis* **27**(7), 465–481 (2022).
47. Roy, A., Das, S., Chatterjee, I., Roy, S. & Chakraborty, R. Anti-inflammatory effects of different dietary antioxidants. In *Plant Antioxidants and Health* 1–25 (Springer, 2022).
48. Üstündağ, H., Kara, A., Doğanay, S. et al. Molecular mechanisms of resveratrol and its silver nanoparticle conjugate in addressing sepsis-induced lung injury. *Naunyn Schmiedeberg's Arch. Pharmacol.* 1–13 (2024).
49. Perez-Serna, A. A. et al. BCL-XL overexpression protects pancreatic β -cells against cytokine- and palmitate-induced apoptosis. *Int. J. Mol. Sci.* **24**(6), 5657 (2023).
50. Šrámek, J., Němcová-Fürstová, V. & Kovář, J. Molecular mechanisms of apoptosis induction and its regulation by fatty acids in pancreatic β -cells. *Int. J. Mol. Sci.* **22**(8), 4285 (2021).

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Declarations

Competing interests

The authors declare no competing interests.

Additional information

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