Journal of Experimental Pharmacology

Dovepress
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ORIGINAL RESEARCH

Effect of Vulgarin and Epivulgarin on Ischemia–Reperfusion-Induced Pancreatic Injury in Rats: A Biochemical, Molecular, and Histopathological Evaluation

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Introduction: Ischemia/reperfusion (I/R) injury is a critical complication associated with pancreatic surgery, and transplantation, which frequently develops into acute pancreatitis due to increased oxidative stress and inflammatory cascades.

Aim: This study aimed to assess the protective effects of vulgarin (VLG) and epivulgarin (EPV) against pancreatic I/R- injury in rats.

Methods: Rats were given oral dosages of 10 or 20 mg/kg of VLG or EPV for two days prior to I/R and 24 h after reperfusion. Pancreatic I/R was induced by occluding the pancreatic blood supply for 60 minutes followed by reperfusion. Key biochemical markers including serum amylase, lipase, tumor necrosis factor-alpha (TNF-α), interleukin-1β (IL-1β), malondialdehyde (MDA), and glutathione peroxidase (GPx) were measured. Additionally, pancreatic tissue expression of high mobility group box 1 (HMGB1) and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) was assessed via immunohistochemistry.

Results: Pancreatic I/R significantly elevated serum levels of amylase (4.5-fold) and lipase (5.8-fold), oxidative stress marker; MDA (8.5-fold), as well as pro-inflammatory cytokines; TNF-α (10.4-fold) and IL-1β (5.6-fold) compared to controls ($p \leq 0.01$). The antioxidant GPx activity was suppressed by 84% ($p \leq 0.05$). Treatment with VLG and EPV dose-dependently attenuated these changes, with the highest dose of EPV reducing serum amylase by 72% and MDA by 76% compared to untreated I/R rats ($p \leq 0.01$). Immunohistochemical analysis revealed marked downregulation of HMGB1 and NF-κB expression in VLG and EPV-treated groups. High-dose therapies showed near normalization of numerous biochemical and molecular parameters.

Conclusion: Vulgarin and epivulgarin exhibited significant protective effects against pancreatic I/R injury by reducing oxidative stress and suppressing HMGB1/NF-κB-mediated inflammatory signaling. These findings suggest the therapeutic potential of VLG and EPV in managing acute pancreatitis and related pancreatic injuries.

Keywords: pancreatic injury, splenic artery occlusion, vulgarin, epivulgarin, HMGB1/NF-κB pathway, rats

Introduction

Ischemia/reperfusion injury (I/R) of the pancreas is one of the important clinical complication associated with shock, pancreatic surgery, and pancreas transplantation.[1] Strong evidence from both experimental data as well as clinical investigations indicates that the pancreas is extremely liable to ischemic damage. Although oxygen levels are restored upon reperfusion, a surge in the generation of reactive oxygen species occurs and proinflammatory neutrophils infiltrate ischemic tissues to exacerbate ischemic injury.[2] Experimental models of acute pancreatitis consistently demonstrate early alterations in pancreatic microvascular perfusion, which exacerbate ischemic damage and contribute to the initiation of I/R injury.[3] Several studies have shown that pancreatic I/R injury stimulates systemic inflammatory reactions due to

https://doi.org/10.2147/JEP.S563840
Received: 28 August 2025
Accepted: 19 January 2026
Published: 2 February 2026

increasing the number of white blood cells in the blood, producing oxygen free radicals, and releasing cytokines.[4] During acute pancreatitis (AP), leukocyte activation and proinflammatory cytokine release are not only restricted to local pancreatic damage, but also responsible for the development of systemic inflammatory response syndrome (SIRS) and multiple organ failure.[5] AP can lead to the improper activation of pancreatic enzymes, which may involve in inducing cell death and lung insult via mechanisms involving protein-derived activators.[6] Due to the unavailability of pharmacologic therapies, the treatment of AP is primarily supportive. Several enhancements were attempted for the constituents of supportive therapy; however, to date, there are no approved treatments to suppress the powerful cascade of inflammatory factors associated with AP.[7]

Artemisia is one of the most widespread genera belonging to the family *Asteraceae*. This genus contains several types of terpenes including sesquiterpenoids.[8] Sesquiterpene lactones are one of the major bioactive classes owing to their diverse structures and wide range of biological activities, including anti-inflammatory, cytotoxic, and antiviral properties.[8] Due to their structural heterogeneity and biodiversity, they are potential platform for the discovery of new bioactive compounds.[9,10] The sesquiterpenes vulgarin (VLG) and epivulgarin (EPV) have been reported from several *Artemisia* species, including *A. vulgaris*, after which they were named.[11,12] Other names were given to VLG such as judaicin from *A. judaica* L. and barrelin from *A. barrelieri* Besser.[8] Rather than plant sources, VLG was chemically synthesized by reduction of peroxyvulgarin and obtained by microbial transformation.[13,14]

A. vulgaris has been used by tribal communities in the western Himalayas traditional medicine for the treatment of rheumatism, stomach and liver problems, and sexual disorders.[15] Vulgarin is naturally occurring sesquiterpene lactone isolated from several Artemisia species, including *Artemisia vulgaris*. Previous studies have indicated that VLG possesses cytotoxic,[16] an anti-inflammatory,[17] antidiabetic,[18] and cardiotonic[19] properties, as well as strong central nervous system stimulant, and convulsant poison.[20] The variable biological activities of VLG therefore make it an attractive target for chemo- and biotransformation studies.

In a previous study by Althurwi et al,[18] rats were given daily oral doses of 10 and 20 mg/kg of vulgarin for 8 weeks, and monitoring of the rats throughout the experimental period did not reveal any changes in their behavior or body weight, confirming the safety of using the two doses for an extended period. Ahmed et al[21] also mentioned that the benefits of using the phenolic part of the ethanolic extract of *A. judaica* plant include its high level of safety. They added that the phenolic part of *A. judaica* can be used to treat water systems. Further, compounds of *A. judaica*; apigenin and quercetin; displayed selective cytotoxic activity against many types of cancer cell lines with low or no toxicity to normal cells.[22]

Most current experimental strategies for reducing pancreatic I/R injury concentrate on single-target interventions like supplementing with antioxidants, inhibiting the activation of digestive enzymes, and suppressing inflammatory mediators. Although a few natural substances have shown some usefulness in lab experiments, none of them have yet been developed into successful therapeutic interventions. While sesquiterpene lactones extracted from *Artemisia* species have demonstrated antioxidant and anti-inflammatory properties in various experimental models, no previous studies have investigated the effects of VLG and EPV on pancreatic I/R injury. Therefore, this study was designed to investigate, for the first time, the possible protective effects of VLG and EPV against I/R-induced pancreatic injury in rats. By addressing the role of VLG and EPV in this previously unexplored area, our findings will expand the current understanding of therapeutic strategies used in pancreatic ischemia and reperfusion injury.

Materials and Methods

Plant Material

The aerial parts of *Artemisia judaica* L. were collected from the Huraymila region, approximately 85 km West of Riyadh City, in January 2018. Plant materials were identified by Dr. Mohammad Atiqur Rahman, a taxonomist at the MAP-PRC, College of Pharmacy, King Saud University. A voucher specimen (#16723) was kept at the herbarium of the center. Details of extraction and isolation of VLG and EPV were described earlier.[18]

Animals

The protocol for the animal study was approved by the Ethical Committee for Medical Research at the National Research Center in Egypt. Thirty-six adult male Wistar rats (180–200 g each) were secured from the animal facility at the National Research Center, Egypt. The animals were placed in standard cages under controlled room temperature, normal dark-light cycles, and pathogen-free conditions. Animals were supplied with standard food and water *ad libitum*. The rats were kept for one week before beginning the experimental protocol to adapt to these conditions. The experiments were performed in compliance with the National Regulations of Animal Welfare and the Institutional Animal Ethical Committee (IAEC; Approval no. 2416072022).

Kits and Chemicals

Biochemical parameters were analyzed using commercially available kits. Amylase was measured using the Rat AMY2 ELISA Kit (Amylase Alpha 2, Pancreatic; Cat. No. NBP2-68207, Novus Biologicals, Centennial, CO, USA). Lipase was determined with the Rat Pancreatic Lipase ELISA Kit (Colorimetric; Cat. No. NBP2-81258, Novus Biologicals, Centennial, CO, USA). Trypsinogen activation peptide (TAP) was quantified using the Rat TAP ELISA Kit (Cat. No. LS-F22914, LifeSpan Biosciences, Inc., Seattle, WA, USA). Lipid peroxidation was assessed by the Lipid Peroxidation (MDA) Colorimetric/Fluorometric Assay Kit (Cat. No. K739-100, BioVision, Milpitas, CA, USA). Glutathione peroxidase activity was measured using the GPx Activity Colorimetric Assay Kit (Cat. No. K762-100, BioVision, Milpitas, CA, USA). Myeloperoxidase (MPO) was determined with the Rat MPO ELISA Kit (Catalog No. E4581-100, BioVision, Milpitas, CA, USA). Tumor necrosis factor-alpha (TNF-α) was quantified using the Rat TNF-α ELISA MAX™ Deluxe Set (Cat. No. 438205, BioLegend, San Diego, CA, USA). Interleukin-1 beta (IL-1β) was measured with the Rat IL-1β ELISA Kit (Cat. No. SEA563Ra, Cloud-Clone Corp., Houston, TX, USA). NF-κB/p65 was analyzed using the NF-κB/p65 ActivELISA™ Kit (Cat. No. NBP2-29661, Novus Biologicals, Centennial, CO, USA). All other chemicals used in this study were of the highest available analytical grade.

Experimental Design

The 36 selected male rats were randomly categorized into 6 equal groups:

Negative control (NC) and Pancreatic IR groups: Rats were subjected to I/R of the pancreas and treated with vehicles.

VLG-10 and VLG-20 groups: Rats treated with VLG at 10 and 20 mg/kg, respectively.

EPV-10 and EPV-20 groups: rats treated with 10 or 20 mg/kg EPV, respectively.

The doses were chosen based on previous pharmacological studies of vulgarin (VLG) and related sesquiterpene lactones, which demonstrated biological activity and safety within this range in rodent models.[18] The treatments were administered orally for 2 days. One-hour after the final dose was administered, the animals were subjected to the procedure for induction of pancreatic ischemia.

Induction of Pancreatic Ischemia

The rats were anesthetized with ketamine (50 mg/kg, intraperitoneally) 60 min after the last dose of the compound was administered. A midline abdominal incision was made to expose the splenic artery. A microvascular clamp was used to occlude the splenic artery for 60 min. Ischemia was confirmed by pancreatic fading of the pancreas.[23] Sixty min later, pancreatic reperfusion was visually observed after the clamp. Silk sutures were used to suture the wound the animals were allowed to recover.[5] Sham-operated group encountered the same procedures but without I/R induction.

Twenty-four hours after reperfusion, another oral dose was administered, and blood samples were obtained via the retro-orbital venous plexus to determine the serum biochemical parameters. Blood was centrifugated at 1538 × *g* for 10 min to separate serum. The rats were executed by decapitation and the pancreas of each rat was separated and cut into two parts. One part was fixed in 10% formalin buffer for 24 h for histological and immunohistochemical (IHC) examination. The other part was dissected and divided into two portions. The first portion was immediately snap-frozen in liquid nitrogen and then stored at −80°C for gene expression analysis while the second portion was immediately

homogenized in ice-cold 10% (w/v) phosphate buffer. The homogenate was centrifuged at 1800 × *g* for 10 min at 4°C. The supernatants were used for biochemical analysis.

Serum Analyses

Serum trypsinogen activated peptide (TAP), amylase, and lipase were analyzed according to the manufacturer's procedures of the used kit.

Assessment of Oxidative Stress and Inflammatory Markers in Pancreatic Tissues

Various inflammatory and oxidative stress markers were measured in the pancreatic homogenates utilizing ELISA technique, such as: myeloperoxidase (MPO), malondialdehyde (MDA), glutathione peroxidase (GPx), tumor necrosis factor-alpha (TNF-α), nuclear factor-kB (NF-κB), and interleukin-1 beta (IL-1β) following the manufacturer's protocol.

Quantitative Analysis of HMGB1 Expression in Pancreas

RNA was extracted from pancreatic tissues and reverse transcribed using TRIzol reagent and Superscript II RNase H Reverse Transcriptase (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol. Gene expression of pancreatic HMGB was quantitatively analyzed using real-time polymerase chain reaction (qRT-PCR), as previously described using the primers specific to HMGB1 and GAPDH as the reference gene.[24,25]

Histopathological Examination of Pancreatic and Liver Tissues

The pancreatic tissues were fixed in 10% neutral-buffered formalin for 24 h. The tissues were then washed with tap water; serial dilutions of alcohol were applied and thereafter the specimens were cleared in xylene for dehydration and embedded in paraffin wax in hot air oven at 56°C for 6 h. The obtained paraffin wax tissue blocks were sectioned by microtome adjusted at 5-6-micron thickness. Sections were collected on glass slides and deparaffinized. They were then stained for routine histological examination using Hematoxylin and Eosin stain.[26]

Immunohistochemical Examination of iNOS in Pancreatic Tissues

Paraffin sections were placed on positively charged slides using the avidin–biotin–peroxidase complex (ABC) method. The. Sections from each group were incubated with the rabbit iNOS polyclonal antibody (Genetex, Cat No. GTX130246, dilution: 1:100) and the required reagents (Vectastain ABC-HRP kit, Vector Laboratories) for the ABC method were added. Marker expression was tagged with peroxidase and stained using diaminobenzidine (DAB, produced by Sigma) to reveal the antigen-antibody complex. Negative controls using non-immune serum instead of primary or secondary antibodies. IHC-stained pancreatic tissue sections were examined with help of an Olympus microscope (BX-53). Scoring was achieved by counting the percentage of the reaction areas using ImageJ 1.53 K software (National Institute of Health, USA).

Statistical Analysis

Before performing statistical analysis, normality was checked using the Shapiro test, and heteroscedasticity was checked using the Brown-Forsythe test. All the data were displayed as mean ± SEM. One-way analysis of variance (one-way ANOVA) followed by Tukey's test were used for statistical analysis to determine the intergroup variability by applying Graph Prism®. Statistical significance was allocated at $p \leq 0.05$.

Results

Assessment of Serum Pancreatic Enzymes

As presented in Table 1, serum pancreatic enzymes were markedly higher in the pancreatic IR group compared with the negative control (NC) group. Specifically, there was a significant increase in serum amylase, lipase, and trypsinogen-activated peptide (TAP) levels in the IR group by 3.52, 4.89, and 7.0 folds compared to the NC group. VLG- and EPV-treated groups showed significantly normalized serum levels of pancreatic enzymes in a dose-dependent manner. VLG-10

Table 1 Effect of Vulgarin and Epivulgarin on the Serum Pancreatic Enzymes of Pancreatic I/R Rats

Group	Amylase (ng/mL)	Lipase (pg/mL)	TAP (ng/mL)
NC	5.0±0.46[b]	24.1±2.29[b]	0.4±0.04[b]
Pancreatic IR	22.6±0.79[a]	142.1±5.49[a]	3.2±0.12[a]
VLG-10	13.4±1.01[a,b]	87.1±4.72[a,b]	2.0±0.08[a,b]
VLG-20	6.6±0.66[b]	54.4±2.91[a,b]	1.1±0.09[a,b]
EPV-10	12.8±0.58[a,b]	90.3±4.29[a,b]	2.0±0.06[a,b]
EPV-20	6.3±0.37[b]	38.1±2.69[b]	1.0±0.07[a,b]

Notes: Values are expressed as Mean ± SEM of six animals in each group. [a]Significantly different from the values of the negative control rats at $p \leq 0.05$. [b]Significantly different from the values of pancreatic IR control rats at $p \leq 0.05$.
Abbreviations: NC, negative control; IR, Ischemia/reperfusion; VLG, Vulgarin; EPV, Epivulgarin.

and VLG-20 significantly reduced serum amylase levels by 40.7% and 70.8%, respectively, when compared to the pancreatic IR group. In contrast, EPV-10 and EPV-20 significantly reduced serum amylase levels by 43.3% and 72.1%, respectively, compared to the pancreatic IR group. Serum lipase was significantly decreased in the VLG-10 and VLG-20 treated groups by 38.7% and 61.7%, respectively, compared to that in the pancreatic IR group. However, serum lipase levels were significantly decreased in the EPV-10 and EPV-20 treated groups by 36.5% and 73.2%, respectively, compared to those in the pancreatic IR group. Serum TAP was significantly decreased in the VLG-10 group by 37.5% and significantly decreased in the VLG-20 group by 65.6% compared to that in the pancreatic IR rats. Furthermore, the EPV-10 and EPV-20 treated groups significantly reduced serum TAP levels by 37.5% and 68.8%, respectively, compared to the pancreatic IR group.

Assessment of Biomarkers of Oxidative Stress Markers in Pancreatic Tissues

Table 2 shows the effects on oxidative biomarkers in pancreatic tissues. The level of pancreatic MDA was markedly elevated in the ischemia/reperfusion (IR) group relative to the normal control (NC) group ($p \leq 0.05$). Conversely, the activities of pancreatic GPx and MPO were notably reduced in the IR group compared to the NC group ($p \leq 0.05$). VLG-10 and VLG-20 treated groups revealed significant decreases in pancreatic MDA levels of 35.3% and 64.7%, respectively, compared to the pancreatic IR control group. Similarly, EPV-10 and EPV-20 significantly decreased MDA levels in the pancreatic tissue by 41.1% and 76.5%, respectively, when compared to those in the pancreatic IR control group. The activity of pancreatic GPx was significantly enhanced by 2.3 and 4.0 folds in the VLG-10 and VLG-20 treated groups, respectively, compared with that in the pancreatic IR control group. Furthermore, the EPV-10 and EPV-20 treated

Table 2 Effect of Vulgarin and Epivulgarin on the Pancreatic Oxidation Markers of Pancreatic I/R Rats

Group	MDA (nmol/mg Protein)	GPx (nmol/mg Protein)	MPO (ng/mg Protein)
NC	0.2±0.02[b]	3.7±0.19[b]	6.3±0.26[b]
Pancreatic IR	1.7±0.06[a]	0.6±0.03[a]	0.9±0.08[a]
VLG-10	1.1±0.06[a,b]	2.0±0.11[a,b]	3.5±0.13[a,b]
VLG-20	0.6±0.06[a,b]	3.0±0.14[a,b]	5.7±0.20[b]
EPV-10	1.0±0.04[a,b]	1.9±0.09[a,b]	3.6±0.16[a,b]
EPV-20	0.4±0.02[b]	3.5±0.14[b]	5.9±0.25[b]

Notes: Values are expressed as Mean ± SEM of six animals in each group. [a]Significantly different from the values of the negative control rats at $p \leq 0.05$. [b]Significantly different from the values of pancreatic IR control rats at $p \leq 0.05$.
Abbreviations: NC, negative control; IR, Ischemia/reperfusion; VLG, Vulgarin; EPV, Epivulgarin.

groups revealed a significant increase in pancreatic GPx activity by 2.2 and 4.8 folds, respectively, compared with the pancreatic IR control group. Moreover, the activity of pancreatic MPO significantly increased in the VLG-10 and VLG-20 treated groups by 2.9 and 5.3 folds, respectively, compared to that in the pancreatic IR control. EPV-10 and EPV-20 treated groups revealed a significant increase in pancreatic MPO activity by 3.0 and 5.6 folds, respectively, compared to the pancreatic IR control.

Assessment of Inflammatory Markers in Pancreatic Tissue

Pancreatic tissue was investigated to assess inflammatory markers Table 3. Pancreatic TNF-α, IL-1β and NF-κB contents were significantly elevated in pancreatic IR control group compared to NC control ($p \leq 0.05$). The VLG and EPV-treated groups showed significantly decreased levels of inflammatory markers in the pancreas when compared to the pancreatic IR group. TNF-α in the pancreas was significantly decreased by 41.8%, 81.7%, 63.5%, and 81.9% in VLG-10, VLG-20, EPV-10, and EPV-20, respectively, compared to pancreatic IR control. Moreover, Pancreatic IL-1β was found to be significantly decreased by 34.6%, 71.0%, 32.4%, and 73.1% in VLG-10, VLG-20, EPV-10, and EPV-20, respectively compared to the pancreatic IR control. Likewise, the pancreatic content of NF-κB was significantly decreased by 43.1%, 78.7%, 50.1% and 86.4% in VLG-10, VLG-20, EPV-10, and EPV-20, respectively, compared to pancreatic IR controls.

Gene Expression Analyses

To explore whether the effects of VLG and EPV on pancreatic I/R injury were related to the regulation of HMGB1, an RT-PCR assay was performed. The results revealed that rats subjected to pancreatic I/R showed a significant ($p \leq 0.05$) increase in HMGB1 expression in the pancreatic tissue compared to the NC group (Figure 1). By contrast, HMGB1mRNA expression showed a significant downregulation in VLG treated groups (VLG-10 and VLG-20) as compared to the pancreatic I/R group (Figure 1). Similar trends were observed in the EPV-treated groups (EPV-10 and EPV-20) (Figure 1).

Histopathological Examination of Liver Tissues

Figure 2 illustrates that the liver tissue of NC rats exhibited normal histological features. In contrast, pancreatic IR led to portal vein congestion, mononuclear inflammatory cell infiltration in the portal region, activation of Kupffer cells, and vacuolar degeneration in some hepatocytes. In the VLG-10 group, hepatic sinusoids appeared dilated with sparse infiltration of mononuclear inflammatory cells among hepatocytes, while the VLG-20 group showed dilated sinusoids along with activated Kupffer cells.

Rats treated with EPV-10 showed mononuclear inflammatory cell infiltration in the portal area, with vacuolar degeneration in some hepatocytes and activation of Kupffer cells, whereas the EPV-20 group showed dilated hepatic sinusoids with activation of Kupffer cells and low mononuclear inflammatory cell infiltration in the hepatic sinusoids.

Table 3 Effect of Vulgarin and Epivulgarin on the Pancreatic Inflammatory Markers of Pancreatic I/R Rats

Group	TNF-α (pg/mg Protein)	IL-1β (pg/mg Protein)	NF-κB (ng/mg Protein)
NC	30.9 ±1.04[b]	38.1±2.64[b]	29.2±1.40[b]
Pancreatic IR	321.2±7.77[a]	212.7±4.17[a]	286.3±15.45[a]
VLG-10	186.8 ±10.82[a,b]	139.0±10.98[a,b]	162.8±9.43[a,b]
VLG-20	58.5±4.11[b]	61.6±1.68[b]	60.7±2.78[b]
EPV-10	117.0±7.27[a,b]	143.7±6.60[a,b]	142.8±7.24[a,b]
EPV-20	58.1±3.49[b]	57.1±4.55[b]	38.7±2.00[b]

Notes: Values are expressed as Mean ± SEM of six animals in each group. [a]Significantly different from the values of the negative control rats at $p \leq 0.05$. [b]Significantly different from the values of pancreatic IR control rats at $p \leq 0.05$.
Abbreviations: NC, negative control; IR, Ischemia/reperfusion; VLG, Vulgarin; EPV, Epivulgarin.

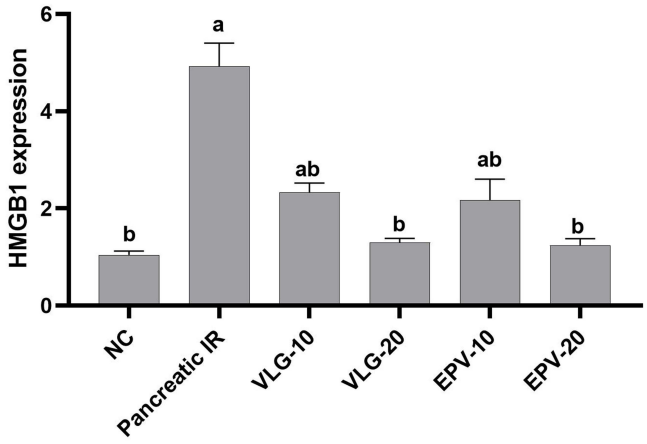

Figure 1 Effect on HMGB1 gene expression in the pancreas. [a]Significantly different from the values of the negative control rats at $p \leq 0.05$. [b]Significantly different from the values of pancreatic IR control rats at $p \leq 0.05$.

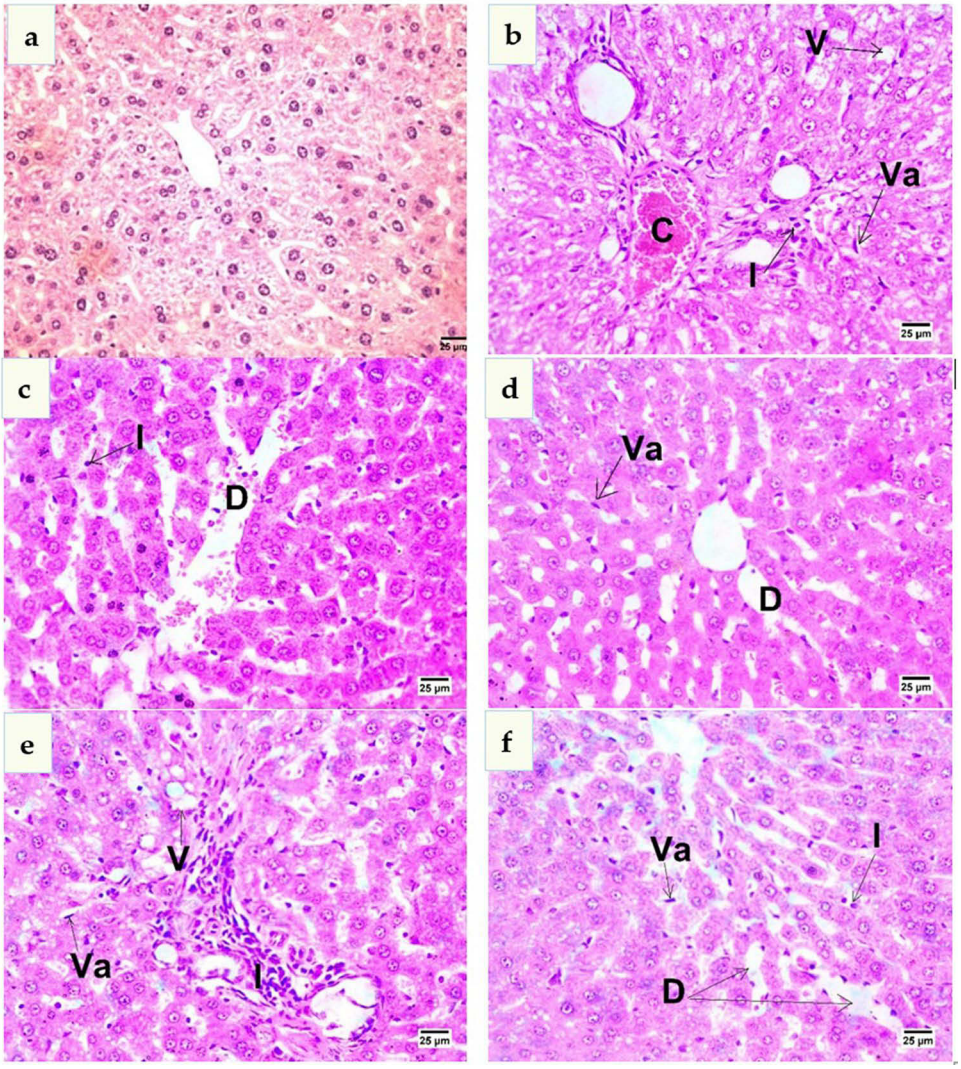

Figure 2 Effect of VLG, EPV on liver damage in pancreatic IR (H&E). (**a**) Photomicrograph of the NC group displaying the normal liver histoarchitecture; (**b**) Pancreatic IR group showing portal vein congestion (C), limited infiltration of mononuclear inflammatory cells in the portal region (I), activation of Kupffer cells (Va), and vacuolar degeneration in some hepatocytes (V); (**c**) VLG-10 group showing dilated hepatic sinusoids filled with blood (D) and sparse mononuclear cell infiltration (I) between hepatocytes; (**d**) VLG-20 group showing sinusoidal dilation (D) and activated Kupffer cells (Va); (**e**) EPV-10 group demonstrating mononuclear cell infiltration in the portal region (I), vacuolar degeneration in some hepatocytes (I), and Kupffer cell activation (Va); (**f**) EPV-20 group showing dilated hepatic sinusoids (D), Kupffer cell activation (Va), and low infiltration of mononuclear inflammatory cells within the sinusoids (I).

https://doi.org/10.2147/JEP.S563840

Histopathological Examination of Pancreatic Tissues

Figure 3 revealed that induction of pancreatic IR resulted in edema with hemorrhage between pancreatic acini. VLG-10 treated group exhibited hemorrhage between pancreatic acini with mononuclear inflammatory cells infiltration, while VLG-20 treated group showed some edema between pancreatic acini. Furthermore, the EPV-10 group showed congestion of the pancreatic blood vessels with edema between the acini. The EPV-20 group showed edema between pancreatic acini.

Immunohistochemical Examination of iNOS in Pancreatic Tissue

As demonstrated in Figures 4 and 5, immunohistochemical staining of pancreatic tissues from the NC group exhibited no detectable expression of inducible nitric oxide synthase (iNOS). In contrast, the pancreatic tissues from rats subjected to ischemia/reperfusion (IR) injury showed strong nuclear iNOS immunoreactivity within the acinar cells. Treatment with VLG resulted in a moderate level of iNOS expression, predominantly localized in the cytoplasm of the pancreatic acini. Meanwhile, administration of EPV led to a relatively mild cytoplasmic iNOS signal in the acinar cells.

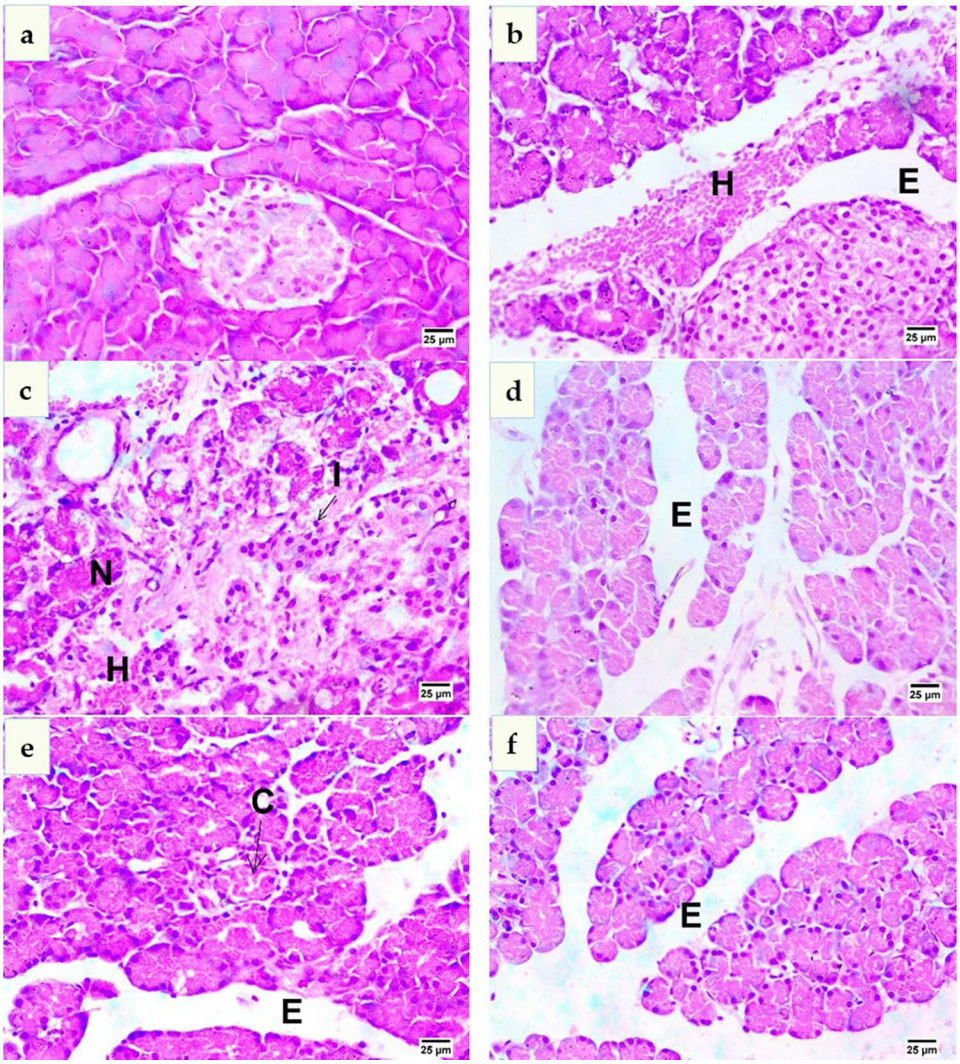

Figure 3 Effect of VLG, EPV on pancreatic damage in pancreatic IR (H& E). (**a**) NC group, photomicrograph showing the normal histological structure of a pancreas; (**b**) pancreatic IR group, photomicrograph showing edema (E) with hemorrhage (H) between pancreatic acini; (**c**) VLG-10 group, photomicrograph showing hemorrhage (H between pancreatic acini with mononuclear inflammatory cells infiltration (I), some pancreatic acini showing necrobiotic changes (N); (**d**) VLG-20 group, photomicrograph showing edema (E) between pancreatic acini; (**e**) EPV-10 group, photomicrograph showing congestion of pancreatic blood vessels (C) with edema between acini (E); (**f**) EPV-20 group, photomicrograph showing edema (E) between pancreatic acini.

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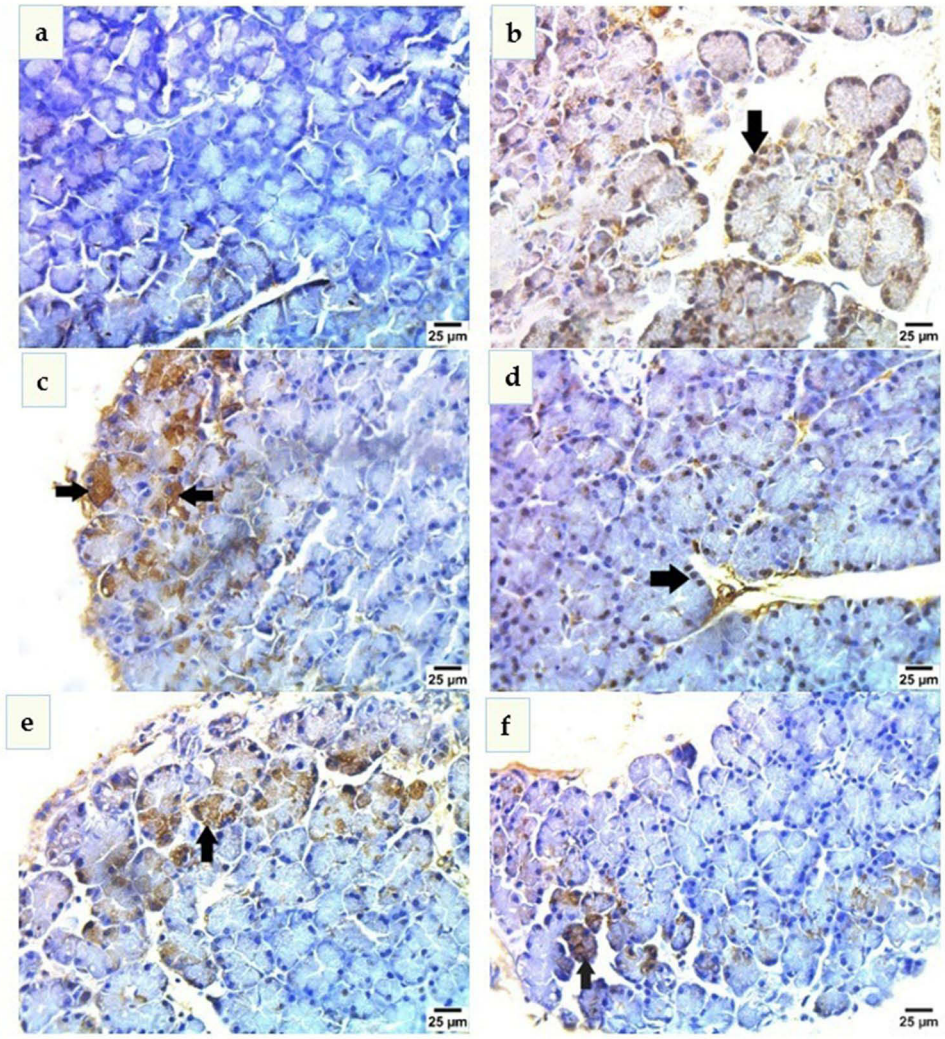

Figure 4 Effect of VLG, EPV on pancreatic tissue iNOS expression in pancreatic IR (IHC-peroxidase-DAB). (**a**) NC group, photomicrograph showing negative reaction for iNOS in pancreatic acini; (**b**) pancreatic IR group, photomicrograph showing strong positive reaction for iNOS in nuclei of pancreatic acini (arrow); (**c**) VLG-10 group, photomicrograph showing moderate positive reaction for iNOS in cytoplasm of pancreatic acini (arrows); (**d**) VLG-20 group, photomicrograph showing moderate positive reaction for iNOS in nuclei of pancreatic acini (arrow; (**e**) EPV-10 group, photomicrograph showing mild positive reaction for iNOS in cytoplasm of pancreatic acini (arrow); (**f**) EPV-20 group, photomicrograph showing very mild positive reaction for iNOS in cytoplasm of pancreatic acini (arrow).

Discussion

Ischemia-reperfusion (I/R) injury is a well-recognized pathological process that extends beyond the pancreas and affects multiple organs, underscoring its systemic relevance. In the heart, reperfusion following myocardial ischemia triggers oxidative stress and NF κB-mediated inflammation, contributing to cardiomyocyte death and adverse remodeling.[27] Similarly, hepatic I/R injury during liver surgery or transplantation involves Kupffer cell activation and HMGB1 release, which amplify inflammatory cascades and tissue damage.[27] In the kidney, I/R is a major cause of acute kidney injury, and studies have shown that renal I/R can even elicit secondary injury in distant organs such as the liver through systemic inflammatory responses.[28] The brain is also highly vulnerable, where reperfusion after stroke exacerbates neuronal death via ROS overproduction and inflammatory signaling.[29] These parallels highlight that the mechanisms observed in pancreatic I/R injury, oxidative stress, cytokine release, and HMGB1/NF κB pathway activation are consistent across organs, reinforcing the broader significance of our findings and suggesting that compounds such as vulgarin and epivulgarin may hold therapeutic potential in diverse I/R related pathologies.

https://doi.org/10.2147/JEP.S563840

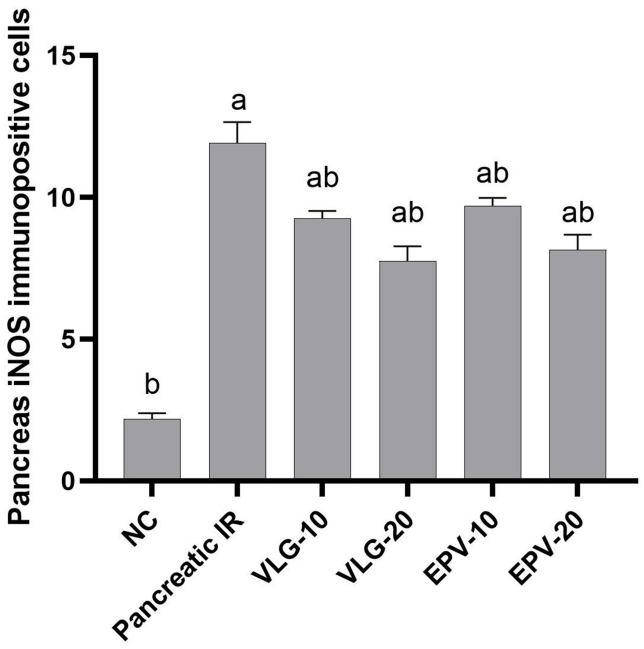

Figure 5 Diagram showing iNOS immunopositive cells. Statistical analysis using one-way ANOVA followed by Tukey–Kramer multiple comparisons test. (a vs NC group, b vs pancreatic IR group) at p≤0.05.

The current study shows that VLG and EPV significantly protect rats from pancreatic ischemia-reperfusion injury. We demonstrated that transient splenic artery occlusion followed by reperfusion causes severe pancreatic damage in a rat model. This damage is marked by elevated serum pancreatic enzymes, an imbalance in oxidative stress, heightened inflammatory responses, and noticeable histopathological changes. Crucially, these pathological alterations were significantly reduced by treatment with VLG and EPV, especially at larger doses.

Acute pancreatitis is known to be triggered by pancreatic I/R damage due to improper activation and leakage of digesting enzymes from the pancreatic acinar cells.[30] Three digestive enzymes generated from pancreatic acinar cells have been considered as biochemical indicators of AP; serum amylase is the most often utilized enzyme in clinical practice. The other two enzymes are lipase and proenzyme trypsinogen.[31] Our findings showed significant increases in serum amylase, lipase, and trypsinogen-activated peptide after I/R insult, indicating acinar cell damage and enzyme activation, in line with previous reports.[23,32]

Notably, treatment with VLG and EPV produced a dose-dependent corrective effect, with the high-dose groups restoring amylase, lipase, and trypsinogen levels close to baseline values. This normalization underscores the potent protective capacity of VLG and EPV against I/R-induced pancreatic damage, particularly at higher doses, indicating successful acinar cell integrity maintenance. These outcomes align with previous studies reporting that extracts derived from *Artemisia judaica* exert inhibitory effects on key carbohydrate-digesting enzymes, including pancreatic α-amylase, intestinal α-glucosidase, and dipeptidyl peptidase-4. Such enzymatic suppression has been associated with notable blood glucose-lowering activity.[33]

The development and progression of numerous I/R-induced injuries are heavily influenced by oxidative stress through the overproduction of ROS.[34] In I/R-induced pancreatitis, excessive ROS production is driven by either injured pancreatic cells or activated immune cells that significantly infiltrate damaged pancreatic tissue.[35] In this investigation, pancreatic I/R significantly raised MDA levels, a well-established indicator of lipid peroxidation and suppressed the enzymatic activities of GPx and MPO in the pancreatic tissues. Interestingly, the administration of VLG and EPV alleviated I/R-induced oxidative stress by reducing pancreatic lipid peroxidation and restoring GPx and MPO activities, indicating a potent antioxidative capacity. These outcomes are in line with Saeedan et al, who proposed that *A. judaica* extracts, which contain VLG and EPV, resulted in oxidative stress reduction.[36]

Inflammation is a major factor in worsening pancreatic damage.[37] The findings from our study demonstrated a substantial increase in the levels of key pro-inflammatory mediators; TNF-α, IL-1β and NF-κB in the pancreatic I/R group. However, the pancreatic TNF-α, IL-1β and NF-κB contents were significantly decreased in a dose dependent manner in groups treated with VLG and EPV compared to the pancreatic I/R group. Excessive release of inflammatory cytokines stimulates macrophage recruitment inflammatory infiltration and into the affected tissues.[38] Subsequently, the activated macrophages produce HMGB1, which amplifies inflammatory signaling through the feedback loop regulation of other cytokines.[39] Meanwhile, the levels of pro-inflammatory cytokines (TNF-α and IL-1β) were decreased in the VLG and EPV-treated groups. These results demonstrate the anti-inflammatory potential of VLG and EPV.

Many statistically significant values observed in the high-dose VLG and EPV groups (especially EPV group) did not differ from those in the NC group. These results demonstrate the ability of high doses of both compounds to reduce the disturbances caused by I/R and to restore several serum biochemical markers (amylase enzyme), pancreatic oxidation markers (MPO), and pancreatic inflammatory markers (TNF-α, IL-1β, NF-κB) to near-normal levels after I/R injury. This normalization suggests a strong protective effect of VLG and EPV and supports the dose-response relationship interpretation.

HMGB1 is a nuclear protein that binds to DNA, recognized as a key endogenous danger signal, and classified as a damage-associated molecular pattern (DAMP) that plays a central role in initiating and propagating inflammatory responses during tissue injuries, such as I/R, hemorrhagic shock, trauma, or sepsis.[40,41] Intracellular and extracellular HMGB1 have opposing roles. Intracellular HMGB1 acts as an important mediator of DNA-associated activities such as transcription, replication, and repair.[42] By contrast, extracellular HMGB1, once released into the intercellular space under stress conditions, shows cytokine-like features and mediates inflammatory responses.[43] This inflammatory role is mediated by the binding of HMGB1 to cell surface receptors such as Toll-like receptors (TLRs) and receptors for advanced glycation end products (RAGE). HMGB1 activates TLR4 leading to induction of a series of signal transduction such as NF-κB pathway.[44]

The NF-κB signaling and its upstream factors such as TLRs and MyD88 have been shown to play essential roles in inflammatory injuries.[45] Upon exposure to oxidative stress, NF-κB releases and translocate into the nucleus to induce the genes encoding IL-1β, IL-6, TNF-α, iNOS and COX-2 protein, which play crucial roles in the inflammatory response including pancreatitis.[46,47] Various previous studies have reported a correlation between HMGB1upregulation and NF-κB pathway activation in pancreatic injuries, and its relationship with the severity of the injury. Therefore, modulation of HMGB1 can provide an effective pharmacological approach for managing pancreatic injury.[48]

Our findings indicate a marked upregulation of HMGB1 expression in pancreatic tissues of the ischemia/reperfusion (I/R) group, accompanied by an increased serum level of NF-κB. This molecular cascade appears to stimulate the activation of Nrf2, a key transcription factor involved in cellular defense, which in turn contributes to the suppression of apoptotic pathways and promoting resistance to oxidative stress. These results align with earlier studies that have reported the involvement of the HMGB1/NF-κB axis in mediating inflammatory and oxidative mechanisms underlying acute pancreatic injury.[49,50]

These results were further supported by histopathological and immunohistochemical studies. Pancreatic edema, bleeding, inflammatory cell infiltration, and iNOS expression—a major factor in stress and inflammatory injury—were all reduced by VLG and EPV. Notably, high-dose treatments consistently showed better protective benefits, supporting the biological significance of the reported results and demonstrating a clear dose-response connection.

Several previous studies have shown that protection against I/R injury occurs through multiple mechanisms, such as reducing oxidative stress, inhibiting apoptosis, and modulating early cellular stress responses. In this connection, Kazak et al[51] reported that different doses of proanthocyanidin administration can effectively protect against testicular damage associated with torsion/detorsion-induced ischemia/reperfusion in rats by preventing oxidative stress and apoptosis. Similarly, Cougnon et al[52] demonstrated that the anti-ischemic effects of the deoxyhypusine synthase inhibitor; GC7 in epithelial cells from the kidney proximal tubule are due to several pathways such as improved antioxidant defences, a metabolic shift and a downregulation of mitochondrial activity. These modulations occur early after treatment and preserve organ function after I/R. Further, Kirgiz et al[53] mentioned that eucalyptol can effectively reduce histopathological damage, apoptosis, and oxidative stress in testicular T/D induced I/R injury. The beneficial effects of eucalyptol can be attributed to its ability to support anti-oxidant systems, block oxidative stress, prevent apoptosis, and protect testicular integrity.

In line with these previously described mechanisms, the results of the current study demonstrated that VLG and EPV exert similar protective effects in pancreatic I/R injury, while providing additional mechanistic insight through the marked downregulation of HMGB1/NF-κB–mediated inflammatory signaling.

From a clinical standpoint, pancreatic damage linked to postoperative pancreatitis, and graft malfunction after pancreatic surgery and transplantation are all significantly influenced by pancreatic ischemia–reperfusion injury. According to the current findings, controlling oxidative stress and HMGB1/NF-κB-driven inflammatory signaling with VLG and EPV may be a sensible therapeutic approach to prevent early pancreatic damage in these situations. Targeting this pathway may have greater translational implications because HMGB1 and NF-κB are known mediators of sterile inflammation in humans.

Study Limitations

Despite these encouraging results, the study has some limitations. One such limitation is the lack of assessment of apoptosis. The absence of assays such as TUNEL staining or caspase-3 activity limits conclusions regarding cell death pathways in I/R injury. In addition, although our findings suggest a potential link between HMGB1/NF-κB signaling and Nrf2 activation, direct evaluation of Nrf2 nuclear translocation and downstream antioxidant gene expression (eg, HO-1, NQO1) was not performed. Future investigations should address these aspects to provide a more comprehensive mechanistic understanding.

Conclusions

In conclusion, results of our study provide the first experimental evidence that VLG and EPV protect against pancreatic ischemia and reperfusion injury. The results show the potential clinical significance of VLG and EPV as modulators of important inflammatory pathways linked to human pancreatic I/R injury. The protective effects of VLG and EPV are likely mediated through the attenuation of oxidative damage and suppression of inflammatory responses. Specifically, the mechanisms appear to involve downregulation of key inflammatory pathways, particularly the HMGB1/NF-κB axis, alongside a reduction in the release of critical pro-inflammatory mediators such as TNF-α, IL-1β, and iNOS. While VLG and EPV emerge as promising candidates for mitigating IR-induced pancreatic damage, further experimental studies on their mechanism of action and safety are needed before clinical application can be considered.

Funding

This research was funded by the Deanship of Scientific Research at Prince Sattam bin Abdulaziz University under the project number (PSAU/2025/03/32788).

Disclosure

The author(s) report no conflicts of interest in this work.

References

1. Chen CF, Chen HT, Wang D, Li JP, Fong Y. Restrictive ventilatory insufficiency and lung injury induced by ischemia/reperfusion of the pancreas in rats. *Transplant Proc*. 2008;40:2185–2187. doi:10.1016/j.transproceed.2008.07.093
2. Kalogeris T, Baines CP, Krenz M, Korthuis RJ. Cell biology of ischemia/reperfusion injury. *Int Rev Cell Mol Biol*. 2012;298:229–317. doi:10.1016/B978-0-12-394309-5.00006-7
3. Hoffmann TF, Leiderer R, Waldner H, et al. Ischemia reperfusion of the pancreas: a new in vivo model for acute pancreatitis in rats. *Res Exp Med*. 1995;195:125–144. doi:10.1007/BF02576782
4. Chen KH, Chao D, Liu CF, et al. Curcumin attenuates airway hyperreactivity induced by ischemia-reperfusion of the pancreas in rats. *Transplant Proc*. 2010;42:744–747. doi:10.1016/j.transproceed.2010.03.017
5. Schanaider A, de Carvalho TP, de Oliveira Coelho S, et al. Ischemia–reperfusion rat model of acute pancreatitis: protein carbonyl as a putative early biomarker of pancreatic injury. *Clin Exp Med*. 2015;15:311–320. doi:10.1007/s10238-014-0289-2
6. Mitsuoka H, Kistler EB, Schmid-Schönbein GW. Generation of in vivo activating factors in the ischemic intestine by pancreatic enzymes. *Proc Natl Acad Sci U S A*. 2000;97:1772–1777. doi:10.1073/pnas.97.4.1772
7. Makhija R, Kingsnorth AN. Cytokine storm in acute pancreatitis. *J Hepatobiliary Pancreat Surg*. 2002;9(4):401–410. doi:10.1007/s005340200049
8. Sary HG, Khedr MA, Orabi KY. Novel vulgarin derivatives: chemical transformation, in silico and in vitro studies. *Molecules*. 2023;28:3421. doi:10.3390/molecules28083421
9. Dong S, Tang J-J, Zhang C-C, et al. Semisynthesis and in vitro cytotoxic evaluation of new analogues of 1-O-acetylbritannilactone, a sesquiterpene from Inula britannica. *Eur J Med Chem*. 2014;80:71–82. doi:10.1016/j.ejmech.2014.04.028

10. Singh R, Mandrah K, Asati A, et al. Transformation of santonin to a naproxen analogue with anti-inflammatory activity. *J Nat Prod*. 2019;82:1710–1713. doi:10.1021/acs.jnatprod.8b00318
11. Abegaz B, Camps F, Coll J, et al. The structures of vulgarin and its isomers. *Tetrahedron*. 1986;42:6003–6009. doi:10.1016/S0040-4020(01)96084-3
12. Geissman TA, Ellestad GA. Vulgarin, a sesquiterpene lactone from *Artemisia vulgaris* L. *J Org Chem*. 1962;27:1855–1859. doi:10.1021/jo01052a092
13. Al-Said MS, Khalifa SI, El-Feraly FS, et al. Biogenetic-type synthesis of vulgarin and peroxyvulgarin. *Phytochemistry*. 1989;28:107–108. doi:10.1016/0031-9422(89)85018-6
14. Arias JM, Bretón JL, Gavín JA, et al. Microbial transformations of sesquiterpenoids: conversion of deoxyvulgarin by *Rhizopus nigricans* and *Aspergillus ochraceous*. *J Chem Soc Perkin Trans*. 1987:471–474. doi:10.1039/P19870000471
15. Khan AU, Gilani AH. Antispasmodic and bronchodilator activities of *Artemisia vulgaris* are mediated through dual blockade of muscarinic receptors and calcium influx. *J Ethnopharmacol*. 2009;126:480–486. doi:10.1016/j.jep.2009.09.010
16. Lee KH, Huang ES, Piantadosi C, et al. Cytotoxicity of sesquiterpene lactones. *Cancer Res*. 1971;31:1649–1654.
17. Zafra-Polo MC, Blázquez MA. Antiinflammatory activity of sesquiterpene lactones from Artemisia barrelieri in rats. *Phytother Res*. 1991;5:91–93. doi:10.1002/ptr.2650050212
18. Althurwi HN, Soliman GA, Abdel-Rahman RF, et al. Vulgarin, a sesquiterpene lactone from *Artemisia judaica*, improves the antidiabetic effectiveness of glibenclamide in streptozotocin-induced diabetic rats via modulation of PEPCK and G6Pase genes expression. *Int J Mol Sci*. 2022;23:15856. doi:10.3390/ijms232415856
19. Galal EE, Kandil A, Abdel-Latif MA, et al. Cardiac pharmaco–toxicological studies of JUDAICIN, isolated from *Artemisia judaica*. *Planta Med*. 1974;25:88–91. doi:10.1055/s-0028-1097918
20. Khafagy SM, Tosson S. Crystallographic, optical and chromatographic studies of judaicin, bitter principle of *Artemisia judaica* L. *Planta Med*. 1968;16:446–449. doi:10.1055/s-0028-1099933
21. Ahmed SA, Eltamany EE, Nafie MS, et al. Anti- Cryptosporidium parvum activity of *Artemisia judaica* L. and its fractions: in vitro and in vivo assays. *Front Microbiol*. 2023;14:1193810. doi:10.3389/fmicb.2023.1193810
22. Goda MS, Nafie MS, Awad BM, et al. In vitro and in vivo studies of anti-lung cancer activity of *Artemesia judaica* L. crude extract combined with LC-MS/MS metabolic profiling, docking simulation and HPLC-DAD quantification. *Antioxidants*. 2022;11(1):17. doi:10.3390/antiox11010017
23. Abdel-Kader MS, Abdel-Rahman RF, Soliman GA, et al. Oleuropein relieves pancreatic ischemia reperfusion injury in rats by suppressing inflammation and oxidative stress through HMGB1/NF-κB pathway. *Int J Mol Sci*. 2024;25:10171. doi:10.3390/ijms251810171
24. Saeedan AS, Abdel-Rahman RF, Soliman GA, et al. Amentoflavone attenuates oxidative stress and neuroinflammation induced by cerebral ischemia/reperfusion in rats by targeting HMGB1-mediated TLR4/NF-κB signaling pathway. *Saudi Pharm J*. 2023;31:101798. doi:10.1016/j.jsps.2023.101798
25. Soliman GA, Alamri MA, Abdel-Rahman RF, et al. Tephrosia purpurea, with (-)-pseudosemiglabrin as the major constituent, alleviates severe acute pancreatitis-mediated acute lung injury by modulating HMGB1 and IL-22. *Int J Mol Sci*. 2025;26:2572. doi:10.3390/ijms26062572
26. Bancroft JD, Gamble M. *Theory and Practice of Histological Techniques*. 6th ed. Elsevier Health Sciences; 2008.
27. Zhao L, Wang Q. Progress in reperfusion injury of other important organs in cardiovascular events. In: *Sudden Death: Advances in Diagnosis and Treatment*. Singapore: Springer Singapore; 2020:93–101.
28. Ziada AA, Kabbash A, El-Aasr M, et al. LC-MS/MS metabolomics approach for chemical characterization of *Beta vulgaris* subspecies maritima extract through untargeted molecular networking, in correlation to its renoprotective activity in renal ischemia/reperfusion induced injury model in rats. *Food Biosci*. 2024;59:104004. doi:10.1016/j.fbio.2024.104004
29. Albaqami FF, Abdel-Rahman RF, Althurwi HN, et al. Targeting inflammation and oxidative stress for protection against ischemic brain injury in rats using cupressuflavone. *Saudi Pharm J*. 2024;32(1):101933. doi:10.1016/j.jsps.2023.101933
30. Logsdon C. Phosphatidylinositol 3-kinase and trypsin activation in pancreatitis. *J Clin Invest*. 2001;108(9):1267–1268. doi:10.1172/JCI14272
31. Matull WR, Pereira SP, O'donohue JW. Biochemical markers of acute pancreatitis. *J Clin Pathol*. 2006;59:340–344. doi:10.1136/jcp.2002.002923
32. Maduzia D, Ceranowicz P, Cieszkowski J, et al. Pretreatment with Warfarin attenuates the development of ischemia/reperfusion-induced acute pancreatitis in rats. *Molecules*. 2020;25(11):2493. doi:10.3390/molecules31010099
33. Bhat SH, Ullah MF, Abu-Duhier FM. Bioactive extract of *Artemisia judaica* causes in vitro inhibition of dipeptidyl peptidase IV and pancreatic/intestinal enzymes of the carbohydrate absorption cascade: implication for anti-diabetic new molecular entities (NMEs). *Orient Pharm Exp Med*. 2019;19:71–80. doi:10.1007/s13596-018-0347-5
34. Gezer A, Üstündağ H, Özkaraca M, et al. Therapeutic effects of resveratrol and β-carotene on L-arginine-induced acute pancreatitis through oxidative stress and inflammatory pathways in rats. *Sci Rep*. 2024;14:32068. doi:10.1038/s41598-024-83764-y
35. Pérez S, Pereda J, Sabater L, et al. Redox signaling in acute pancreatitis. *Redox Biol*. 2015;5:1–14. doi:10.1016/j.redox.2015.01.014
36. Saeedan AS, Soliman GA, Abdel-Rahman RF, et al. *Artemisia judaica* L. diminishes diabetes-induced reproductive dysfunction in male rats via activation of Nrf2/HO-1-mediated antioxidant responses. *Saudi J Biol Sci*. 2021;28:1713–1722. doi:10.1016/j.sjbs.2020.12.013
37. Gukovsky I, Li N, Todoric J, et al. Inflammation, autophagy, and obesity: common features in the pathogenesis of pancreatitis and pancreatic cancer. *Gastroenterology*. 2013;144(6):1199–1209. doi:10.1053/j.gastro.2013.02.007
38. Hickey FB, Martin F. Role of the immune system in diabetic kidney disease. *Curr Diab Rep*. 2018;18:20. doi:10.1007/s11892-018-0984-6
39. Gan Z-S, Wang -Q-Q, Li J-H, et al. Iron reduces M1 macrophage polarization in RAW264.7 macrophages associated with inhibition of STAT1. *Mediators Inflamm*. 2017;2017:8570818. doi:10.1155/2017/8570818
40. Kang R, Chen R, Zhang Q, et al. HMGB1 in health and disease. *Mol Aspects Med*. 2014;40:1–116. doi:10.1016/j.mam.2014.05.001
41. Abdelmageed ME, Nader MA, Zaghloul MS. Targeting HMGB1/TLR4/NF-κB signaling pathway by protocatechuic acid protects against l-arginine induced acute pancreatitis and multiple organs injury in rats. *Eur J Pharmacol*. 2021;906:174279. doi:10.1016/j.ejphar.2021.174279
42. Martinotti S, Patrone M, Ranzato E. Emerging roles for HMGB1 protein in immunity, inflammation, and cancer. *ImmunoTargets Ther*. 2015;4:101–109. doi:10.2147/ITT.S58064
43. Yuan J, Guo L, Ma J, et al. HMGB1 as an extracellular pro-inflammatory cytokine: implications for drug-induced organic damage. *Cell Biol Toxicol*. 2024;40:55. doi:10.1007/s10565-024-09893-2
44. Zhong H, Li X, Zhou S, et al. Interplay between RAGE and TLR4 regulates HMGB1-induced inflammation by promoting cell surface expression of RAGE and TLR4. *J Immunol*. 2020;205:767–775. doi:10.4049/jimmunol.1900860

45. Su Q, Lv X, Sun Y, et al. Role of TLR4/MyD88/NF-κB signaling pathway in coronary microembolization-induced myocardial injury prevented and treated with nicorandil. *Biomed Pharmacother*. 2018;106:776–784. doi:10.1016/j.biopha.2018.07.014
46. Saad HM, Elekhnawy E, Shaldam MA, et al. Rosuvastatin and diosmetin inhibited the HSP70/TLR4 /NF-κB p65/NLRP3 signaling pathways and switched macrophage to M2 phenotype in a rat model of acute kidney injury induced by cisplatin. *Biomed Pharmacother*. 2024;171:116151. doi:10.1016/j.biopha.2024.116151
47. Ali BM, Al-Mokaddem AK, Selim HM, et al. Pinocembrin's protective effect against acute pancreatitis in a rat model: the correlation between TLR4/NF-κB/NLRP3 and miR-34a-5p/SIRT1/Nrf2/HO-1 pathways. *Biomed Pharmacother*. 2024;176:116854. doi:10.1016/j.biopha.2024.116854
48. Yang R, Tenhunen J, Tonnessen TI. HMGB1 and histones play a significant role in inducing systemic inflammation and multiple organ dysfunctions in severe acute pancreatitis. *Int J Inflam*. 2017;2017:1817564. doi:10.1155/2017/1817564
49. Wu X, Yang Z, Wang H, et al. High-mobility group box protein-1 induces acute pancreatitis through activation of neutrophil extracellular trap and subsequent production of IL-1β. *Life Sci*. 2021;286:119231. doi:10.1016/j.lfs.2021.119231
50. Li G, Wu X, Yang L, et al. TLR4-mediated NF-κB signaling pathway mediates HMGB1-induced pancreatic injury in mice with severe acute pancreatitis. *Int J Mol Med*. 2016;37:99–107. doi:10.3892/ijmm.2015.2410
51. Kazak F, Akcakavak G, Alakus I, et al. Proanthocyanidin alleviates testicular torsion/detorsion-induced ischemia/reperfusion injury in rats. *Tissue Cell*. 2024;89:102459. doi:10.1016/j.tice.2024.102459
52. Cougnon M, Giraud S, Materozzi M, et al. Inhibition of eIF5A hypusination enhances antioxidant defense to prevent kidney Ischemia/Reperfusion injury. *Redox Biol*. 2015;86:103814. doi:10.1016/j.redox.2025.103814
53. Kirgiz O, Akcakavak F, Akcakavak G, et al. Eucalyptol attenuates testicular ischemia-reperfusion injury in rats. *Vet Res Forum*. 2025;16 (11):611–620. doi:10.30466/vrf.2025.2048928.4593