Protective effect of *Glycyrrhiza glabra* L. root (licorice) extract against severe acute pancreatitis-induced acute lung injury via suppressing autophagy and inflammation

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ARTICLE INFO	**ABSTRACT**
	Introduction: Acute pancreatitis (AP) is an inflammatory disease with a high incidence of morbidity and mortality rate. The present study aimed to evaluate the protective effect of licorice extract administration on L-arginine-induced AP and associated lung tissue damage in rats. **Methods:** The experimental groups were the healthy control group (G1), L-arginine group (G2), licorice extract group (G3), and licorice extract +L-arginine; (protection group; G4). The protective effect of licorice extract was evaluated by measuring serum amylase and lipase, oxidative stress markers (malondialdehyde, nitric oxide, and myeloperoxidase), and inflammatory biomarkers levels (interleukin-6, tumor necrosis factor-alpha, toll-like receptor 4, and vascular cell adhesion molecule 1), as well as apoptosis assessment via caspase-3 activity and beclin-1 expression. Furthermore, an immunohistochemical assessment of heme oxygenase-1 (HO-1) and a histopathological examination of lung and pancreatic tissues were performed. **Results:** Licorice extract administration significantly reduced serum amylase, lipase, and inflammatory markers levels that pointed to the local and systemic inflammatory condition of AP induced by L-arginine. Moreover, the administration of licorice extract reversed the significant elevation in oxidative stress markers levels in the pancreas and lung tissues. Furthermore, licorice extract downregulated pancreatic gene expression of beclin-1 and caspase-3 which reversed dysregulated pancreatic autophagy. **Conclusion:** Licorice extract administration causes modulation of oxidative damage and systemic inflammation associated with acute pancreatic damage. Moreover, licorice extract markedly decreases the biochemical and histopathologic changes in AP, preserving the pancreatic and lung tissues through its antioxidant, anti-inflammatory, and antiapoptotic effects.

Introduction

Acute pancreatitis (AP) is an inflammatory disease with a high morbidity and mortality rate. This disorder is characterized by a complicated etiology, rapid progression, and poor prognosis (1). AP has a complex etiology, including chronic alcohol consumption, genetic factors, bad dietary habits, gallstones, and obesity. The most common complication of AP is acute lung injury.

Moreover, repeated bouts of AP may result in the loss of pancreatic tissue function and fibrosis. The pathogenesis of AP includes various mechanisms such as oxidative stress, induction of proinflammatory mediators, dysregulated autophagy, and disrupted microcirculation (2).

Oxidative stress is one of the main pathogenic factors of AP, provoking cytokine storm and systemic inflammatory response syndrome (3). Furthermore, the overproduction

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of ROS is associated with hyperamylasemia and intra-acinar activation of digestive pancreatic enzymes. Oxidative damage results in lysosomal/autophagic dysfunction, systemic inflammation, and pancreatic acinar cell necrosis (1). Moreover, deregulated autophagy triggers an inflammatory response in the pancreas. The induction of proinflammatory mediators results in inflammatory cell infiltration in the pancreas, systemic inflammatory response, and acinar cell death through apoptosis and necrosis (4). Furthermore, the systemic inflammatory response associated with pancreatic injury results in multiorgan failure.

Acute lung injury is reported as the most common distant organ disease in AP and the main cause of AP mortality. Acute lung injury results from pulmonary microvascular endothelial cell damage caused by the activation of inflammatory pathways (5).

Natural herbs play a crucial role in modern drug development. This era has shown a substantial interest in medicinal plants for their therapeutic role in several acute and chronic diseases. Many studies have reported that plant phytochemical compounds combat inflammatory cytokines storm and oxidative damage. Licorice root (*Glycyrrhiza glabra*) or sweet wood belongs to the family *Fabaceae*. Since ancient times licorice has been widely used for its pharmacological and medicinal effects. It is widely cultivated in Egypt, Asia, and Europe; glycyrrhizin and glycyrrhetinic acid are the main bioactive components isolated from licorice root (6). Licorice extract possesses various pharmacological activities, including antioxidative, anti-inflammatory, cardioprotective, antiviral, antimicrobial, antidiabetic, anticancer, immunomodulatory, and hepatoprotective activities (7).

Licorice root contains more than 300 phytochemicals including triterpenes, saponins, flavonoids, isoflavonoids, and chalcones. The chalcones have been reported to contribute for the pharmacological effects of licorice extract. Remarkably, the synergistic actions of licorice extract components provide a more potent anti-inflammatory effect (8). The present study was conducted to investigate the effect of oral administration of licorice root extract on AP and lung tissue injury induced by L-arginine. In this study, we aim to evaluate the effects of licorice root extract on the suppressing oxidative and proinflammatory cascade pathways and dysregulated autophagy in AP and lung injury.

Materials and Methods
Materials
Dried licorice root (*G. glabra*) was obtained from the Ministry of Agriculture, Cairo University (Giza, Egypt) (Herbarium number 191). The L-arginine powder was obtained from STC Company in Egypt.

Experimental animals
Thirty-two adult male Wistar rats, weighing 200± 5 g, were maintained in separate cages on a 12-hour light/dark cycle. The temperature was maintained at 25°C and humidity between 60% and 70%. The rats were fed standard rat chow pellets according to (9) and water *ad libitum* during the acclimatization period.

Methods
1. Preparation of licorice extract
The dried licorice roots were ground into a powder with a blender (Philips, Japan). About 500 g of the ground roots were soaked in 1 L of the hydroalcoholic mixture (30% water and 70% ethanol) for 72 hours in a dark place as described by Srikantam and Arumugam (8) with slight modification. After filtration, the extraction solvent was evaporated using a rotary evaporator at 40°C, and the residue obtained was then weighed.

2. Preparation of L-arginine dose
The L-arginine powder was prepared by dissolving in 0.9% saline solution at a concentration of 500 mg/mL and the pH was adjusted to 7 using 0.1 M (0.1 N) sodium hydroxide (NaOH) solution according to previous research (10).

3. Experimental design
Licorice extract was administered daily via intragastric intubation at a dose of 400 mg/kg (11). The L-arginine was injected twice at 2.5 g/kg intraperitoneally (i.p.) at a one-hour interval (2). Animals were grouped as follows:
- Group 1 (control group): Rats were fed a standard commercial diet and injected with normal saline i.p.
- Group 2 (L-arginine injected group): Rats were fed a standard commercial diet and injected with L-arginine (2 i.p. 1 hour interval) to induce AP.
- Group 3 (licorice root extract group): Rats were fed a standard commercial diet and administered licorice extract (p.o.) daily.
- Group 4 (protection group): Rats were fed on a standard commercial diet+ licorice extract (p.o.) daily for 8 successive days, then 24 hours after the last treatment, rats were injected with L-arginine (2 i.p. 1 hour interval) for induction of AP.

Samples collection
At the end of the experiment, rats were anesthetized under sodium pentobarbital at 200 mg/kg. Blood sample was collected from the hepatic portal vein, its serum was separated and kept at -20°C until biochemical analysis. The pancreas and lung tissues were dissected immediately. The first portion of the pancreas and lung tissues were stored at -20°C for the estimation of biochemical parameters. Another portion of both tissues was fixed in 10% formalin and paraffin embedded for histological analysis. The third

portion of the pancreas was stored at -80°C for molecular assay using the real-time polymerase chain reaction (PCR) technique and the last portion of the pancreas was fixed for immunohistochemical analysis.

Biochemical analysis

1. Estimation of serum inflammatory markers

1.1. Assessment of serum tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) levels

Serum TNF-α and IL-6 were estimated following the quantitative sandwich enzyme-linked immunosorbent assay (ELISA) technique according to the manufacturer's instructions using cloud-clone corp. kits (USA).

1.2. Assessment of serum Toll-like receptor 4 (TLR 4) and vascular cell adhesion molecule-1 (VCAM-1)

TLR 4 and VCAM-1 assay were performed following the quantitative sandwich ELISA technique according to the manufacturer's instructions using CusaBio kits (USA).

2. Serum amylase and lipase assay

Serum pancreatic lipase and α-amylase enzymes were performed following the ELISA technique according to the methods described by Tsuzuki et al (12) and Winn-Deen et al (13), respectively using CusaBio kits (USA).

3. Tissue analysis

3.1. Preparation of tissue homogenate

The isolated pancreas and lung were washed with ice-cold saline and homogenized in potassium phosphate buffer (0.01 M, pH 7.4) by a homogenizer (Heidolph Diax 900, Germany) at low temperature. Homogenates were centrifuged at 5000 rpm for 10 min at 4°C. Then, the resulting supernatant was used to determine malondialdehyde (MDA), nitric oxide (NO), and myeloperoxidase (MPO) levels.

3.2. Assessment of oxidative stress biomarkers

MDA, NO, and MPO levels were measured in pancreatic and lung tissues according to the ELISA technique described by Ohkawa et al (14), Montgomery and Dymock (15) and Kuebler et al (16), respectively using Cloud-Clone Corp. kits (USA).

3.3. Quantitative gene expression by real-time PCR

Assessment of apoptosis was done via measuring gene expression of caspase-3 activity and beclin-1 by real-time PCR. Total RNA from pancreatic tissues was extracted using TRIzol RNA extraction reagents (Invitrogen, USA). The cDNA was reversely transcribed using a reverse transcription kit (Vazyme, Nanjing, China). Then the relative expression levels of mRNA were detected using SYBR Green qPCR Master Mix (QIAGEN, Germany). The tested genes were beclin-1 and caspase 3; and a PCR detection system (Step One Applied Biosystem, Foster City, USA) was used. The results were statistically analyzed using the $2^{-\Delta\Delta Ct}$ method, with GAPDH as an internal reference (housekeeping gene). Relative quantification of the expressed genes calculated according to Derveaux et al (17).

Table 1 shows primers sequences.

3.4. Microscopic examination of pancreas and lung tissues

Pancreatic and lung tissues were dissected and rapidly fixed in 10% formalin, paraffin-embedded, and stained with a hematoxylin-eosin stain. Then, the stained tissue sections were examined under bright-field microscopy (Olympus Optical, Japan) at 200× magnification.

3.5. Immunohistochemical staining

4-μm-thick sections of formalin-fixed, paraffin-embedded pancreatic tissue were used for immunohistochemical analysis of heme-oxygenase-1 (HO-1). The sections were deparaffinized using xylene and rehydrated in five graded concentrations of ethanol. The sections were rinsed in water and heated in 0.01 M citrate-hydrochloric acid for 15 minutes using a microwave. After blocking non-specific proteins with 5% bovine serum albumin at 37°C for 1.5 hours, HO-1 antibody was applied to sections and incubated at 4°C overnight. After incubation with the peroxidase-conjugated secondary antibodies, staining with diaminobenzidine and counterstaining with hematoxylin were performed. Then, negative controls were replaced with antirabbit immunoglobulins. The sections were observed under a light microscope at ×200 magnification (Olympus, Japan). Microscopic images were assessed using ImageJ software.

Statistical analysis

Statistical analysis of the results was done using analytical software named SPSS statistics 17.0, Chicago, USA. Values were expressed as means ± SD. Quantitative differences between values were statistically analyzed by one-way ANOVA and the mean difference was considered significant at $P < 0.05$ (18).

Results

Effect of oral administration of licorice extract on serum α-amylase and lipase levels in the experimental groups

L-arginine caused a significant increase ($P \leq 0.05$) in

Table 1. Primers sequences

Gene		Primers
Beclin 1	Forward sequence	5′AGTTCGGTGGGGTCATGTGTG3′
	Reverse sequence	5′CCAGGTATGCACCCAGAGTG3′
Caspase 3	Forward sequence	5′AGGACTCTAGACGGCATCCA3′
	Reverse sequence	5′CAGTGAGACTTGGTGCAGTGA3′
GAPDH	Forward sequence	5′TGGATTTGGACGCATTGGTC3′
	Reverse sequence	5′TTTGCACTGGTACGTGTTGAT3′

pancreatic lipase and α-amylase activities as compared to the healthy control group. Compared to the AP control group, serum activities of pancreatic lipase and α-amylase activities were significantly decreased ($P \le 0.05$) in the licorice extract-protection group (Table 2).

Effect of oral administration of licorice extract on serum IL-6 and TNF-α levels in the experimental groups

Results presented in Table 3 show a significant elevation ($P \le 0.05$) in IL-6 and TNF-α in the serum levels of the AP group compared with the healthy control group. Licorice extract administration significantly decreased IL-6 and TNF-α serum levels compared to the L-arginine group.

Effect of oral administration of licorice extract on serum TLR 4 and VCAM-1 levels in the experimental groups

Results presented in Table 4 show a significant elevation ($P \le 0.05$) of TLR 4 as well as VCAM-1 serum levels of the AP group compared with the healthy control group; whereas licorice extract administration significantly decreased TLR 4 and VCAM-1 serum levels compared to the L-arginine group.

Effect of oral administration of licorice extract on pancreatic MDA, NO, and MPO levels in the experimental groups

L-arginine caused a significant elevation in MDA, NO, and MPO levels compared to the healthy control group ($P < 0.05$). Licorice extract administration caused a significant reduction in MDA, NO, and MPO levels when compared to the AP group (Table 5).

Effect of oral administration of licorice extract on lung MDA, NO, and MPO levels in the experimental groups

Results shown in Table 6 show that concerning MDA, NO, and MPO levels in lung tissue, the AP control group showed a significant increase ($P \le 0.05$) in MDA, NO, and MPO of lung tissue levels as compared to the healthy control group. However, in comparing the licorice extract protection group with the AP control group a significant decrease ($P \le 0.05$) was observed.

Effect of oral administration of licorice extract on caspase 3 and beclin-1 gene expression in pancreatic tissue of the experimental groups

The caspase 3 and beclin-1 mRNA expressions were significantly upregulated ($P \le 0.05$) in the AP control group inducing apoptosis in pancreatic tissues as compared to the healthy control group; whereas licorice extract administration significantly ($P \le 0.05$) downregulated caspase 3 and beclin-1 mRNA expression as compared to the AP control group (Table 7).

Microscopic findings

The obtained figures of the microscopic examination of the pancreas and lung tissues showed that L-arginine induced pancreatic tissue injury and lung tissue damage as a complication of AP; while the licorice extract administration showed marked preservation of pancreatic and lung architecture.

Normal structure of both exocrine units and endocrine

Table 2. Effect of oral administration of licorice extract on serum amylase and lipase levels in the experimental groups

Groups	Parameters	
	Serum amylase (U/L)	Serum lipase (ng/mL)
Healthy control (G1)	29.82 ± 0.96[a]	1.40 ± 0.81[a]
Acute pancreatitis group(G2)	101.99 ± 6.67[b]	6.50 ± 0.32[b]
Licorice group (G3)	28.15 ± 1.51[a]	1.20 ± 0.27[a]
L-arginine+ licorice extract group (G4)	45.89 ± 45.89[c]	2.40 ± 0.31[c]
LSD	3.82	0.28

All values are expressed as mean ± SD (n=10). There is no significant difference between the means that have the same alphabetical superscripts in the same column, while different letters show significantly different ($P < 0.05$).

Table 3. Effect of oral administration of licorice extract on serum IL-6 and TNF-α levels in the experimental groups

Groups	Parameters	
	IL-6 (pg/mL)	TNF-α (pg/mL)
Healthy control (G1)	58.18 ± 2.08 [a]	83.01 ± 2.39 [a]
Acute pancreatitis group(G2)	234.52 ± 25.03[b]	277.18 ±22.27[b]
Licorice group (G3)	53.62 ± 3.08[a]	77.74 ± 1.62[a]
L-arginine+ licorice extract group (G4)	109.42 ± 13.9[c]	127.99±21.10[c]
LSD	14.79	15.77

All values are expressed as mean ± SD (n=10). There is no significant difference between the means that have the same alphabetical superscripts in the same column, while different letters show significantly different ($P < 0.05$).

Table 4. Effect of oral administration of licorice extract on serum TLR 4 and VCAM-1 levels in the experimental groups

Groups	Parameters	
	TLR 4 (ng/mL)	VCAM-1 (ng/mL)
Healthy control (G1)	0.93 ± 0.12[a]	38.07 ± 2.07[a]
Acute pancreatitis group(G2)	7.35 ± 0.61[b]	168.11 ± 7.89[b]
Licorice group (G3)	0.59 ± 0.08[a]	30.72 ± 0.41[c]
L-arginine+ licorice extract group (G4)	4.24 ± 0.72[c]	104.72 ± 11.18[d]
LSD	0.49	7.09

All values are expressed as mean ± SD (n=10). There is no significant difference between the means that have the same alphabetical superscripts in the same column, while different letters show significantly different ($P < 0.05$).

Table 5. Effect of oral administration of licorice extract on pancreatic MDA, NO, and MPO levels in the experimental groups

Groups	Parameters		
	MDA (n. mol/mg)	NO (n. mol/mg)	MPO (ng/mg)
Healthy control (G1)	0.47 ± 0.06 [a]	1.72 ± 0.16 [a]	0.37 ± 0.04[a]
Acute pancreatitis group(G2)	2.77 ± 0.26 [b]	5.95 ± 0.54 [b]	3.21 ± 0.41 [b]
Licorice group (G3)	0.51 ± 0.03 [a]	1.66 ± 0.04 [a]	0.38 ± 0.04 [a]
L-arginine+ licorice extract group (G4)	1.54 ± 0.36 [c]	3.83 ± 0.26 [c]	1.44 ± 0.31 [c]
LSD	0.23	0.32	0.26

MDA, malondialdehyde; NO, nitric oxide; MPO, myeloperoxidase. All values are expressed as mean ± SD (n=10). There is no significant difference between the means that have the same alphabetical superscripts in the same column, while different letters show significantly different ($P < 0.05$).

Table 6. Effect of oral administration of licorice extract on lung MDA, NO, and MPO levels in the experimental groups

Groups	Parameters		
	MDA (n. mol/mg)	NO (n. mol/mg)	MPO (ng/mg)
Healthy control (G1)	0.82 ± 0.03 [a]	1.87 ± 0.08 [a]	0.45 ± 0.07 [a]
Acute pancreatitis group(G2)	4.71 ± 0.60 [b]	9.98 ± 1.59 [b]	5.11 ± 0.13 [b]
Licorice group (G3)	0.76 ± 0.04 [a]	1.85 ± 0.04 [a]	0.69 ± 0.15 [a]
L-arginine+ licorice extract group (G4)	3.39 ± 0.29 [c]	4.62 ± 0.62 [c]	3.67 ± 0.49 [c]
LSD	0.34	0.87	0.27

MDA, malondialdehyde; NO, nitric oxide; MPO, myeloperoxidase. All values are expressed as mean ± SD (n=10). There is no significant difference between the means that have the same alphabetical superscripts in the same column, while different letters show significantly different ($P < 0.05$).

Table 7. Effect of oral administration of licorice extract on caspase 3 and beclin-1 gene expressions in pancreatic tissue of the experimental groups

Groups	Parameters	
	Relative gene expression of (caspase 3/HGAPDH)	Relative gene expression of (beclin-1/HGAPDH)
Healthy control (G1)	1.00 ± 0.00 [a]	1.00 ± 0.00 [a]
Acute pancreatitis group(G2)	4.46 ± 0.01[b]	3.24 ± 0.03[b]
Licorice group (G3)	1.04 ± 0.02[a]	1.19 ± 0.02 [c]
L-arginine+ licorice extract group (G4)	2.42 ± 0.07[c]	2.14 ± 0.03 [d]
LSD	0.11	0.07

All values are expressed as mean ± SD (n=10). There is no significant difference between the means that have the same alphabetical superscripts in the same column, while different letters show significantly different ($P < 0.05$).

components of the pancreas was observed. The islets of Langerhans appeared of normal size and contained β-cells in the healthy control group (G1) (Figure 1a); while the positive control group (G2) showed atrophied ill-distinct islets of Langerhans (Figure 1b). Several sections showed numerous inflammatory cell infiltration between the exocrine acini. Moreover, the peripancreatic adipose tissue showed severe inflammation that was characterized by intense mononuclear inflammatory cell infiltration; whereas the normal histological structure of the pancreatic exocrine and endocrine components was shown in the licorice extract group (G3) (Figure 1c). Furthermore, marked enhancement was observed in the protection group (G4) (Figure 1d) which revealed normal islets of Langerhans in several examined sections. Few sections showed less inflammatory cell infiltration.

On the other hand, a histopathological examination of lung tissue from the healthy control group (G1) (Figure 2a) revealed normal histology of lungs that contained numerous air alveoli and many bronchioles. Adversely, the positive control group (G2) (Figure 2b) showed extensive pulmonary hemorrhages in several examined sections that were characterized by excessive exudation of erythrocytes. The bronchioles showed marked hyperplasia associated with intraluminal accumulation of eosinophilic tissue debris mixed with desquamated epithelial cells. Moreover, peribronchiolar inflammatory cell infiltration was also detected in regarding the G2 group; while in the licorice extract group (G3) a normal histological structure was detected in the examined lung specimens of all rats. The pulmonary tissue showed normal alveoli, bronchi, and bronchioles (Figure 2c). Furthermore, marked improvement was observed in the G4 group. Normal alveoli and bronchioles were detected in several examined
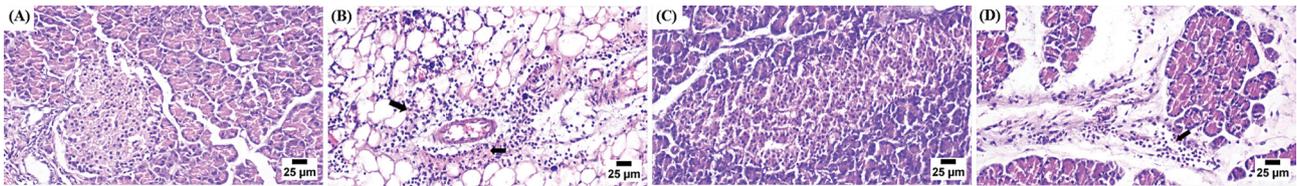
Figure 1. Microscopic examination of pancreatic tissue (H&E). (A) Healthy control group (negative control group; G1) showing normal histology of exocrine acini and normal islets of Langerhans and exocrine acini. (B) L-arginine injected group (positive control group; G2) showing hemorrhages and intense inflammatory cells infiltration in the peripancreatic adipose tissue and severe acute pancreatitis. (C) Licorice extract group; G3, showing normal histology of pancreatic tissue. (D) Protection group; G4, showing fewer inflammatory cells infiltration.
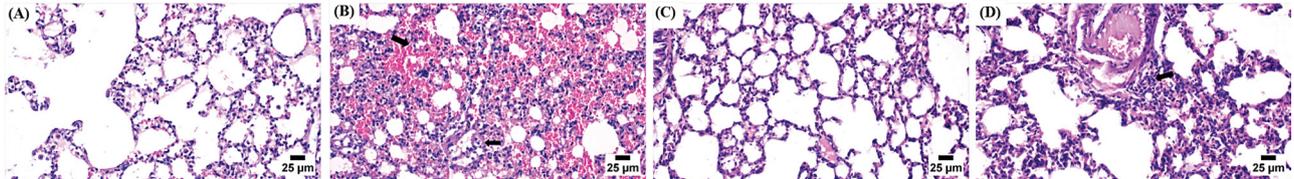

Figure 2. Microscopic examination of lung tissue (H&E). (A) Healthy control group (negative control group; G1), showing a normal structure of bronchiole and surround alveoli, normal lung tissue. (B) L-arginine injected group (positive control group; G2), showing severe pulmonary hemorrhages associated with mononuclear inflammatory cells infiltration. (C) Licorice extract group; G3, showing a normal structure of lung tissue. (D) Protection group; G4, showing fewer perivascular inflammatory cells infiltration.

sections. Mildly congested blood vessels were noticed in some affected areas associated with mild hyperplasia of the bronchial wall and fewer desquamated epithelial cells bronchioles (Figure 2d).

Lastly, the biochemical analysis results obtained were in line with the microscopic observations.

The expression of HO-1 (Figure 3) showed the lowest expression in group 1 (Figure 3a) and group 3 (Figure 3b). Meanwhile, groups 2 (Figure 3c) and 4 (Figure 3d) showed strong positive expression in the pancreatic tissue.

The statistical evaluation of HO-1 area % expression showed no statistical difference ($P < 0.05$) between groups 1 and 3; while group 4 showed a significant increase when compared with other experimental groups. Moreover, when licorice was administered before L-arginine injection, modulatory changes were obvious. It is likely that L-arginine-induced HO-1 downregulation is activated by licorice extract.

Discussion

AP is an acute inflammatory disorder associated with acinar cell injury, upsurge of inflammatory cytokines, systemic inflammatory response syndrome, and acute lung injury. Therefore, the use of natural herbs may be beneficial for the treatment of AP-induced acute lung injury due to their anti-inflammatory activities, decreasing the mortality rate of patients (5).

The objective of this study was to examine the effect of the administration of licorice root extract on modulating AP and lung tissue damage induced by L-arginine. The licorice extract administration attenuated the severity of AP, as indicated by the reduced levels of pancreatic enzymes, inflammatory mediators, and pancreatitis-associated lung injury. In addition, licorice extract administration inhibited pancreatic autophagy and oxidative stress.

Natural herbal medicines gained great interest in the last decades due to their efficacy and minimal side effects. Licorice is widely used in herbal medicines due to its powerful anti-inflammatory and antioxidative properties. *G. glabra* is one of the medicinal plants employed to treat inflammatory diseases since ancient times. The licorice
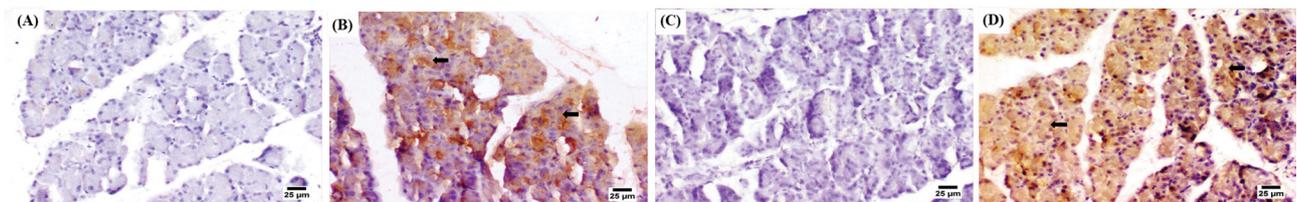

Figure 3. Immunohistochemical results of HO-1 in pancreatic tissue. (A) Healthy control group (negative control group), showing few to weak expression of heme oxigenase-1 (immunostaining). (B) L-arginine injected group (positive control group), showing higher expression of heme oxigenase-1 (immunostaining). (C) Licorice extract group, showing negative expression of heme oxigenase-1 (immunostaining). (D) Protection group, showing strong expression of heme oxigenase-1 (immunostaining).

extract is currently used in the pharmaceuticals and food supplements (19).

Licorice (*G. glabra Linn*) is used in traditional medicine for its promising pharmacological effect. It contains important phytoconstituents such as glycyrrhizin, glabrin A and B, glycyrrhizic acid, and isoflavones. These bioactive compounds have been shown to reduce inflammation and oxidative damage (20).

Oxidative stress is a mediator of autophagic dysfunction in pancreatitis through the generation of ROS as well as, inflammatory cytokines. These mediators contribute to the accumulation of macrophages and neutrophils that trigger a cascade of pathological changes leading to the occurrence and aggravation of AP. Moreover, oxidative and nitrosative stress is implicated in the occurrence of AP and consequent lung injury (21).

L-arginine was reported to induce AP through activation of oxidative stress. The overproduction of NO and MPO activation contributes to the severity and pathogenicity of this disease. Increased ROS production induces inflammatory cell infiltration and lipid peroxidation; while HO-1 activation was reported to counteract the oxidative stimuli (22). This observation was similar to our results and is in line with our histopathological and immunohistochemical findings. Moreover, the administration of licorice extract was shown to significantly reduce the increased levels of MDA, MPO, and NO, modulating oxidative stimuli in pancreatic and lung tissue. Thus, result reflects the antioxidant effect of licorice bioactive components.

The inflammatory condition of AP is first initiated by the activation of various pancreatic enzymes, followed by the release of proinflammatory mediators and acinar cells injury (23). This was also consistent with our results where L-arginine induced increased pancreatic amylase and lipase. AP is associated with a surge in the expression of cytokines and cell adhesion molecules, while licorice administration was reported to decrease the levels of MPO, TNF-α, and IL-1β in pancreatic tissue. Additionally, licorice reduced MDA and serum pancreatic amylase and lipase levels. Thus, licorice inhibits oxidative stress-induced tissue damage and inflammation of pancreatic tissues in experimental pancreatitis model (8). Additionally, lung injury is often reported as the most common cause of early death in AP due to the accumulation of inflammatory cytokines in the lung destroying the blood-air barrier. Several studies have demonstrated that the accumulation of neutrophils in the lungs increased ROS generation, adhesion molecules production with profuse expression of VCAM-1 triggering inflammatory cascade (24); furthermore, the activation of IL-6 and TNF-α in early AP amplifies series of inflammatory cascade. TNF-α activates the VCAM-1 that is critical for the recruitment of leukocytes into the extravascular space of pulmonary microcirculation. Also, some studies concluded that

TLRs, especially TLR-4, is initially activated in AP leading to its further activation of nuclear factor kappa B (NF-κB) (25). Then, the activated TLR-4 and NF-κB provoke pro-inflammatory cytokines synthesis (TNF-α, IL-1β, and IL-6), thus, resulting in persistent and intensified inflammation (24).

Our study showed that, during the AP, there was an obvious increase in serum TLR 4, TNF-α, IL-6, and VCAM-levels. The elevated inflammatory signals concentration induced inflammatory response and were consistent with pathological changes seen in lung and pancreatic tissues. As oxidative stress, inflammatory infiltration is correlated to the histopathological changes. Licorice extract has powerful antioxidant and anti-inflammatory effects. In this study, licorice extract administration was shown to attenuate local and systemic inflammation compared with L-arginine injected control. The elevated levels of serum IL-6, MPO, TNF, TLR-4, and VCAM-1 were significantly reduced by licorice extract administration. The possible synergistic action of the phytochemical mixture present in licorice extract might responsible for its pharmacological activity.

Licorice flavonoids and tannins components inhibited pro-inflammatory signaling mediators. Glycyrrhizin, an anti-inflammatory component of licorice root, inhibited pro-inflammatory cytokines as TNF-α and NO in experimentally induced acute lung injury. Isoliquiritigenin exerted a potent anti-inflammatory effect through suppression of NF-κB activity, consequently attenuated the activation of proinflammatory cytokines (TNF-α, IL-6, IL-1β, and IL-8) (26). In addition, licochalcone E inhibited the NF-κB thus leading to decreased levels of its multiple downstream targets inflammatory signaling, such as NO and VCAM-1. Moreover, isoliquiritigenin significantly decreased the levels of TLR 4 protein and its downstream targets (27).

Autophagy is a multi-step process essential for normal cell homeostasis. Autophagy has been reported to be regulated by oxidative stress and inflammatory cytokines. Deregulated autophagy promotes the inflammatory response in pancreatic tissue. Beclin-1 a multi domain protein plays a significant role in the cross-regulation of autophagy and apoptosis. Moreover, caspases participate in inflammation and cell death; caspases are shown to interact and cause cleavage of several autophagy proteins including beclin-1 and reduce their autophagic function (28).

Glycyrrhizin, a bioactive compound of licorice, was reported to modulate the expression of beclin-1. Thus, phytochemical compounds acting as modulators of beclin-1 could be therapeutically beneficial in AP (27). Moreover, TLR-4 activation triggered apoptosis via a high caspase-3 activity (22), while licorice caused a significant reduction in caspase-3 activity (28). Thus, it is supposed that licorice extract protected the pancreas from

autophagic impairment and protected pancreatic acinar cells from injury. Therefore, manipulating these signals to change the pattern of death responses may act as a potential therapeutic strategy for modulating pancreatitis severity. As oxidative stress is a mediator for autophagic impairment in pancreatitis, licorice antioxidant activity prevented oxidative stress-induced autophagy.

L-arginine induced pancreatic and lung cell injury, inflammatory infiltration, and necrosis (8). In our study, L-arginine caused degenerative changes in pancreatic acini and inflammatory cell infiltration of the pancreas and lung tissue. Histopathological observations showed that licorice root extract administration ameliorated the pathological alterations in tissue architecture in both pancreas and lung, emphasizing its protective effect. Licorice extract flavonoids showed marked anti-inflammatory effects. Isoliquiritigenin possess protective effect against AP through amelioration of oxidative stress and modulating the nuclear factor erythroid 2-related factor/heme oxygenase-1 (Nrf2/HO-1) pathway. Nrf2/HO-1 is a central regulator of cellular antioxidant responses; its activation can alleviate the severity of AP (29). Our results showed that licorice extract activated HO-1 expression thereby activating the antioxidant and anti-inflammatory pathways. Thus, protecting pancreatic tissue from oxidative damage.

Conclusion
This research demonstrated that licorice root extract was able to protect against the local and systemic inflammatory conditions of acute pancreatitis induced by l-arginine. Licorice suppresses dysregulated autophagy and acute lung injury through modulation of the proinflammatory signaling cascade. Furthermore, the possible synergistic action between the complex mixture of phenolic and flavonoid compounds in licorice extract attenuated inflammation and alleviated oxidative stress, regulated autophagy, and preserved pancreatic and lung tissues, thus preventing morbidity.

Authors' contributions
NG and EH designed and performed the experiments and collected the data. NG and EH prepared the manuscript. All authors read and approved the final manuscript for publication.

Conflict of interests
The authors declared no conflict of interest.

Ethical considerations
All authors declare that "Principles of laboratory animal care" were followed. All animal experiments were performed under a protocol approved by the Local Institutional Animal Ethics Committee of Ain Shams University (ASU-SCI/BIOC/2022/12/2).

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