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Hydroalcoholic extract of licorice (*Glycyrrhiza glabra* L.) root attenuates ethanol and cerulein induced pancreatitis in rats

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ABSTRACT

Objective: To evaluate the therapeutic potential of hydroalcoholic extract of licorice root against ethanol and cerulein induced chronic pancreatitis in rats.

Methods: The phytochemical profile of hydroalcoholic extract of licorice root was determined by high performance liquid chromatography (HPLC) and gas chromatography coupled to mass spectrometry (GC-MS). Chronic pancreatitis was induced in male albino Wistar rats by feeding them a diet containing ethanol (0%-36% of total calories) for 4 weeks and cerulein (20 μg/kg b.wt, i.p.) thrice a week for 3 weeks. Lipase and amylase in serum, lipid peroxides and antioxidants including reduced glutathione, glutathione peroxidase, superoxide dismutase and catalase in pancreas were determined. Inflammatory response was measured by myeloperoxidase in the pancreas, caspase-1 and the concentrations of IL-1 β and IL-18 in serum. Moreover, histological evaluation of the pancreas and liver was carried out.

Results: Different flavonoids and saponins were identified in the hydroalcoholic extract of licorice root through HPLC and GC-MS. A marked increase in the levels of serum lipase, amylase, lipid peroxides, caspase-1, myeloperoxidase, IL-1 β , and IL-18 and a marked decrease in the levels of antioxidants were observed after ethanol and cerulein administration. Treatment with hydroalcoholic extract of licorice root attenuated these changes. In addition, histological observation confirmed the protective effect of the extract in the pancreas and liver against inflammatory changes induced by ethanol and cerulein.

Conclusions: The licorice root extract attenuates ethanol and cerulein induced pancreatitis in rats probably due to its antioxidant phytonutrients since ethanol and cerulein-induced production of reactive oxygen species contributes to severe inflammation in the pancreas.

1. Introduction

The pancreas consists of acinar and islet cells which synthesize and secrete digestive enzymes and hormones, respectively. Acute or chronic pancreatitis is characterized by inflammatory reactions that result in the premature intracellular or interstitial activation of trypsinogen causing pancreatic auto-digestion. Acute pancreatitis (AP) is of sudden onset and short duration. Recurrent AP can lead to chronic pancreatitis (CP) which is a progressive inflammatory and fibrotic disease. It develops gradually and worsens over time, resulting in the irreversible weakening of the pancreas, causing pancreatic exocrine and endocrine insufficiencies. CP is a known

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risk factor for pancreatic adenocarcinoma.

CP is associated with severe abdominal pain, maldigestion, malabsorption, steatorrhea, and weight loss. Alcohol abuse, gallstone-related diseases and recurrent AP are found to be the major causes of CP. Alcohol abuse is the primary cause of CP, accounting for nearly 70%–80% of all cases[1]. Alcoholic chronic pancreatitis which affects the quality of life was previously more prevalent in developed countries, but is now increasing worldwide due to increased alcohol consumption[2].

Rats, when chronically administered ethanol, have an increased susceptibility to AP induced by cerulein. Cerulein, an analogue of cholecystokinin, elicits pancreatic injury by hyperstimulation of the exocrine pancreas. The key features of human alcoholic CP are reproduced in the rat with ethanol and repeated doses of cerulein[3]. In pancreatic acinar cells, local inflammation is thus initiated by the release of inflammatory mediators called cytokines (IL-1 β , IL-6, IL-10 and TNF- α) and chemokines. TNF- α is a potent activator of NF- κ B, a pro-inflammatory transcription factor. The initial inflammatory process leads to the recruitment of innate immune cells such as neutrophils and macrophages towards the site of injury. These immune cells play a critical role in the pathogenesis of pancreatitis. Neutrophils increase disease severity by the production of reactive oxygen species (ROS)[4]. These infiltrating cells along with injured acinar cells, in turn, produce more cytokines and chemokines increasing inflammatory response in the pancreas. CP also results in oxidative stress which amplifies the inflammatory process.

Pancreatic acinar cells are known to metabolize ethanol. Ethanol-induced toxicity is mediated mostly through the oxidative and non-oxidative metabolism of ethanol. In oxidative metabolism, alcohol dehydrogenase (ADH) oxidizes ethanol to acetaldehyde followed by conversion of acetaldehyde to acetate by aldehyde dehydrogenase (ALDH), specifically localized in the mitochondrion. Depletion of oxidized nicotinamide adenine dinucleotide (NAD^+) and accumulation of reduced nicotinamide adenine dinucleotide (NADH) take place, as both ADH and ALDH consume NAD^+, producing NADH. Oxidative metabolism of ethanol to acetaldehyde by CYP2E1 produces excessive ROS which also activates NF- κ B which is highly ROS sensitive. In non-oxidative metabolism, fatty acid ethyl ester (FAEE) synthase converts ethanol to FAEE. Thus, ethanol metabolism results in accumulation of acetaldehyde, acetate, NADH and FAEE, as well as changes in cellular and mitochondrial NAD^+ and NADH levels. FAEEs are known to generate a sustained increase in intracellular calcium and subsequent mitochondrial injury, in turn contributing to oxidative stress[5].

Cerulein binds to a G-protein coupled receptor called cholecystokinin receptor on the pancreatic acinar cell membrane and activates phospholipase C and inositol triphosphate-dependent calcium release from the endoplasmic reticulum. This can activate NADPH oxidase which produces ROS, which can disturb the integrity of mitochondria by causing mitochondrial dysfunction. ROS can also cause cytokine signalling through NF- κ B pathway[6]. Increased ROS also results in the oxidation of lipids and proteins and disruption of the pancreatic cell membrane. ROS, proteases and growth factors released by neutrophils and macrophages cause tissue destruction, as well as fibroblast proliferation[7].

The stroma of the pancreas has a clinically important cell type called pancreatic stellate cells (PSCs). In the pancreas, PSCs maintain normal tissue architecture through the synthesis and degradation of extracellular matrix proteins (like collagen). Various pathophysiological changes following pancreatic injury transform quiescent stellate cells into myofibroblast-like cells which actively proliferate and produce more extracellular matrix proteins leading to fibrosis observed in CP. Many scientific reports explained that damage to the pancreatic tissue caused by ethanol and its metabolite acetaldehyde (through MAP kinase pathway), ROS and pro-inflammatory cytokines causes the activation of PSCs. Release of growth factors such as the platelet-derived growth factor, transforming growth factors (TGF- β) secreted by infiltrating cells (monocytes, macrophages) also leads to persistent activation of PSCs[8]. Deng *et al* has reported that chronic ethanol administration increases fibrosis formation in response to cerulein-induced pancreatitis in rats and is highly driven by anti-inflammatory responses to pancreatic injury and/or chronic oxidative stress. So, the recruitment of inflammatory cells provides cytokine signals for PSC activation and fibrosis formation in CP[3].

The major event responsible for the disease severity, progression and cell death in pancreatitis is NF- κ B activation. NF- κ B mediated pathway is central inflammatory pathway that operates in pancreatitis. Damage associated molecular patterns (DAMPs) released from dead and dying pancreatic acinar cells, ROS, acetaldehyde and FAEEs from ethanol metabolism were reported to activate NF- κ B in CP. Ethanol also sensitizes pancreas to the inflammatory response through protein kinase C-mediated pathway of NF- κ B activation[9]. Cerulein causes NF- κ B activation through NADPH oxidase that produces ROS. NF- κ B is kept inactive in the cytoplasm through sequestration in complexes with the inhibitor of NF- κ B (I κ B) proteins. The sequestration prevents NF- κ B migration into the nucleus, its binding to DNA and transcriptional activation. In response to stimuli, the I κ Bs are phosphorylated on specific serine residues by I κ B kinases, resulting in I κ B ubiquitylation and proteasome-mediated degradation. This allows nuclear translocation of NF- κ B, to mediate the expression of cytokines. ROS generated in response to ethanol and cerulein could activate I κ B kinase and NF- κ B[6]. Furthermore, ethanol treatment augments the cerulein-induced activation of NF- κ B.

Pancreatic acinar cell death releases endogenous molecules of the host DAMPs, into the extracellular space that triggers inflammation. Acinar cells and recruited inflammatory cells have pattern recognition receptors to recognise DAMPs. DAMPs mediate the assembly and activation of receptor complexes called inflammasomes. Signalling through pattern recognition receptors like toll like receptor 4 and 9 results in activation of NF- κ B facilitated inflammasome. Different types of inflammasomes are characterized in different organs. NLRP3 is one such inflammasome activated in pancreas in response to cell injury by endogenous and exogenous sources. Activation of NLRP3 inflammasome releases active form of caspase-1, a cytosolic protease which converts pro-

IL-1 β and pro-IL-18 to active IL-1 β and IL-18, serving a central role in the inflammatory response[10]. Oxidative stress and its constant companion persistent inflammation, play critical roles in the pathogenesis of CP and its many complications.

Most therapies center on pain relief with non-steroidal anti-inflammatory drugs (NSAIDs) and management of exocrine and endocrine shortages by supportive therapy. But long-term treatment with NSAIDs can induce pain tolerance, addiction and severe side effects. As there is no single modality treatment approach for CP, it is essential to identify effective and less toxic natural compounds.

Natural products play a crucial role in modern drug development. Many studies reported that plant extracts or plant secondary metabolites such as phenolic compounds, saponins and flavonoids show anti-inflammatory activity. They control the levels of various inflammatory cytokines and their most important regulator, nuclear factor NF- κ B[11]. To exploit such properties, the present study focused on an effective and potential ayurvedic medicinal herb, *Glycyrrhiza glabra* Linn. (*G. glabra*). It is a perennial herb of the pea family (Fabaceae) found widely in Asia and the Mediterranean region. It is commonly known as 'yastimadhu' or licorice. Wide spread use of licorice as demulcent, expectorant and in ulcer healing is well documented in traditions from ancient Assyrian, Egyptian, Chinese and Indian cultures[12].

Licorice roots have been shown to possess anti-inflammatory, anti-oxidative, anti-viral and expectorant properties. They are also found effective in increasing the detoxification capacity of the liver. Phytochemically, licorice roots have shown to contain triterpene saponins, flavonoids, coumarins and phytosterols. The biologically-active components of licorice are glycyrrhizic acid, liquiritin, glabridin and liquiritigenin[13].

This study was conducted to evaluate whether licorice root extract can ameliorate oxidative stress and alter the levels of cytokines responsible for inflammation in the pancreas of male albino Wistar rats administered ethanol and cerulein.

2. Materials and methods

2.1. Chemicals

ELISA kits for caspase-1 and IL-1 β were purchased from Abcam, ELISA kit for IL-18 purchased was from Invitrogen BioServices, India. All the other chemicals and solvents used for the analysis were of analytical grade and were purchased from Merck, India.

2.2. Preparation of plant extract

The licorice roots were bought from the local market (Chennai) and authenticated by Dr. P. Jayaraman, Taxonomist, Plant Anatomy Research Centre, Chennai, Tamilnadu (specimen no. PARC/2016/3303). Surface cleaned roots were shade-dried, pulverized and macerated with 70% ethanol (in water) at room temperature. Maceration was carried out by soaking the pulverized roots in 70% ethanol for 3 d with frequent agitation in a shaker. Then the mixture was strained, pressed and soaked again in ethanol. The maceration step was repeated 3-4 times[14]. Finally, the combined filtrate was clarified by filtration using Whatmann no.1 filter paper, semi air-dried and lyophilized to gain the hydroalcoholic extract of licorice root and stored at 4 ℃ until further analysis. The yield was 12% w/w.

2.3. High performance liquid chromatography (HPLC) analysis of hydroalcoholic extract of licorice root

HPLC analysis was carried out on Shimadzu HPLC CLASS-VP™ series equipped with 2489 UV/Visible detector for the identification and quantitative determination of phytochemicals in hydroalcoholic extract of licorice root. EZChrom Elite Data System was used for data acquisition, processing and control of HPLC and report generation.

2.3.1. Identification and quantification of flavonoids

About 10 g of hydroalcoholic extract of licorice root was refluxed with the extraction solvent - alcohol, water and hydrochloric acid mixture (50:20:8) - on a hot water bath for 135 min (to hydrolyse glycosides to aglycones), then decanted and sonicated for 30 min using methanol. The contents were filtered and diluted to 100 mL with methanol. Quercetin, kaempferol, isorhamnetin, galangin, luteolin, rutin and thymoquinone served as the reference standards. The mobile phase used was a mixture of methanol, water, and phosphoric acid (100:100:1). The samples were run on symmetry C18 column [100 Å pore size, 3.5 μm, 4.6 mm × 250 mm (product no. WAT200632)] with a flow rate of 1.5 mL per minute. Isocratically eluted compounds were detected by UV detector at 270 nm. By matching the retention time and their spectral characteristics against those of the standards, the flavonoids were identified in hydroalcoholic extract of licorice root.

2.3.2. Identification and quantification of saponins

Equal volumes of glycyrrhizin standard and sample (20 μL) were separately injected into the chromatograph and the separation was carried out by isocratic elution. Methanol-water-acetic acid mixture (60:34:6) was the mobile phase used. The flow rate was 1 mL/min through a C-18 reverse phase column. Measurement of UV absorbance was done at 254 nm[15]. By matching the retention time and the spectral characteristics against that of the standard, a saponin was identified in hydroalcoholic extract of licorice root.

2.4. Phytochemical screening by gas chromatography coupled to mass spectrometry (GC-MS)

Hydroalcoholic extract of licorice root was subjected to GC-MS analysis on Agilent 6890N gas chromatograph with HP-5MS column, coupled to a mass spectrometer JEOL GC-MATE Ⅱ in the electron ionization mode with ionization voltage set to 70 eV. The mass spectral scan range of the mass analyser was set to 50-600 amu. Carrier gas used was helium, at a constant flow of 1 mL/min. The GC-oven was set at a ramp rate from 50 ℃-250 ℃ at 10 ℃/min.

Compounds were identified using NIST database (National Institute of Standards and Technology).

2.5. Animals

Male albino Wistar rats weighing 175-200 g were maintained on a 12-hour light-dark cycle at 25 ℃, humidity between 60% and 70%. The rats were housed in polyethylene cages, and given free access to standard rat chow pellets and water during the acclimatization period. Institutional Animal Ethics Committee approved the experimental protocol (XIX/VELS/PCOL/06/2000/CPCSEA/IAEC/03.10.2016).

2.6. Induction of chronic pancreatitis using ethanol and cerulein

After the acclimatization for 1 week, rats were randomly assigned into 4 groups (6 rats in each group). Group 1 (control) and 2 (100 mg/kg body weight of hydroalcoholic extract of licorice root) rats were fed standard rat chow and water *ad libitum* for 5 weeks. Group 3 (ethanol + cerulein) and 4 (ethanol + cerulein + 100 mg/kg body weight of hydroalcoholic extract of licorice root) were fed an isocalorically adjusted diet containing ethanol (0%-36% of total calories) for 4 weeks and subjected to intraperitoneal injection of cerulein (20 μg/kg body weight) 3 times a week for the last three weeks[3]. Group 2 and 4 rats received 100 mg/kg body weight of hydroalcoholic extract of licorice root orally from the third week onwards. Initially, a dose-response study was conducted with different concentrations (25, 50, 100 and 200 mg/kg body weight) of hydroalcoholic extract of licorice root and a dose of 100 mg/kg body weight, which gave the maximum anti-inflammatory activity against pancreatitis was selected for further study.

After 5 weeks, all the animals were fasted overnight, anesthetized using diethyl ether and euthanized by cervical decapitation. Immediately, blood was collected, the serum separated and stored at 4 ℃ until further analyses. Pancreas and liver were removed immediately. A part of these organs from each rat was fixed in 10% formal saline for 24 h and subjected to processing for histopathological examinations.

2.7. Preparation of tissue homogenate

The isolated pancreas was washed with ice cold saline and homogenized in 0.1 M Tris HCl buffer (pH 7.4) at low temperature. The supernatant was used to determine protein, reduced glutathione (GSH), lipid peroxides and the antioxidant enzymes-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx).

2.8. Biochemical investigations

2.8.1. Determination of serum lipase and serum amylase activity

The activity of lipase in serum was assayed according to the method of Lowry and Tinsley[16]. The lipolysis reaction was initiated with the addition of serum in 25 mL olive oil/triton-emulsion as substrate. About 0.3 mL subsamples of the reaction mixture were taken at pre-determined intervals and used for the assay of liberated free fatty acids spectrophotometrically at 715 nm. The activity of the enzyme was expressed as IU/L.

According to the method of Gomori[17], the enzyme activity was determined by measuring the maltose liberated from the starch (substrate) using Lugol's iodine solution. The color intensity was measured spectrophotometrically at 640 nm and the enzyme activity was expressed as IU/L.

2.8.2. Estimation of protein

The protein content of pancreatic homogenate was measured according to the method of Bradford[18] and was used to calculate the specific activities of enzymes.

2.8.3. Estimation of GSH and antioxidant enzymes

GSH level was determined by the method of Moron *et al*[19] and expressed as mg/g protein. GPx was assayed by the method of Flohe and Gunzler[20]. The activity of GPx was expressed as nM of glutathione oxidized/min/mg protein. SOD activity was measured according to the method of Kakkar *et al*[21]. The assay is based on the inhibition of formation of NADH-phenazine-methosulfate nitroblue tetrazolium formazan. The color developed was extracted with *n*-butanol and measured at 560 nm. CAT activity was defined as the amount of enzyme required to decompose 1 μM of H_2O_2 per min. Decomposition of H_2O_2 in the presence of CAT was kinetically measured at 240 nm.The enzyme activity was expressed as μM of H_2O_2 consumed/min/mg protein[22].

2.8.4. Estimation of lipid peroxides

Malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE) are the main products of lipid peroxidation. MDA is a major reactive aldehyde resulting from peroxidation of membrane lipids. TBARS assay was used to determine MDA level[23] and the value was expressed as nM/mg tissue protein.

To estimate 4-HNE, the pancreatic homogenate was allowed to react with 2,4-dinitrophenylhydrazine, mixed thoroughly and set aside for 1 h to react with 4-HNE in the sample. Then the formed adduct of 4-HNE and DNPH was extracted thrice with hexane and evaporated to dryness at 40 ℃. After cooling, 1 mL methanol was added to the residue and the absorbance read at 350 nm spectrophotometrically.

2.8.5. Estimation of inflammatory markers

Myeloperoxidase (MPO), IL-1 β , IL-18 and caspase-1 are the major markers of pancreatic inflammation. MPO was extracted from homogenized tissue and activity was measured according to the method of Bradley *et al*[24]. The change in absorbance after adding the reagents [50 mM potassium phosphate buffer (pH 6.0) containing 0.167 mg/mL o-dianisidine dihydrochloride and 0.000 5% hydrogen peroxide] was measured at 460 nm for 4 min using UV-visible spectrophotometer and expressed as units/mg protein. The assay of IL-1 β was performed according to the manual of Rat IL-1 β ELISA Kit (ab100767) and the activity was expressed as pg/mL. The assay of IL-18 was carried out according to the manual of Rat IL-18 ELISA Kit (KRC2341) and the activity was expressed as pg/mL.The assay of caspase-1 was performed in undiluted serum and suitably diluted pancreatic tissue homogenate according to the procedure

shown in caspase-1 activity assay kit (ab39470). The assay is based on spectrophotometric detection of the chromophore *p*-nitroanilide (*p*-NA) derived from the labeled substrate YVAD-*p*-NA by the activity of caspase-1 present in the sources.

2.9. Histopathological examinations

The fixative was washed from formalin-fixed pancreas and liver tissue samples, dehydrated through a graded series of alcohol, cleaned in methyl benzoate and embedded in paraffin wax. The paraffin-embedded tissue samples were then sectioned (5 μm), stained with hematoxylin and eosin. The slides were then observed under light microscope at 400× magnification.

2.10. Statistical analysis

Data were analysed by the statistics software package (SPSS for Windows v.10). The statistical significance of mean values between different groups was determined by one-way ANOVA with *post hoc* Bonferroni test and P value < 0.05 was considered significant.

3. Results

3.1. HPLC analysis of hydroalcoholic extract of licorice root

3.1.1. Identification and quantification of flavonoids

HPLC-UV chromatogram of the flavonoids in hydroalcoholic extract of licorice root showed the presence of four major peaks at 270 nm with the retention times of 4.527, 11.310, 16.890 and 40.010 min, respectively (Figure 1B). The peaks were identified as rutin, luteolin, thymoquinone and galangin at a concentration of 0.9 mg/g, 0.6 mg/g, 0.5 mg/g, and 1.3 mg/g, respectively. Compounds were confirmed through the comparison of their retention times with those of the standards (Figure 1A).

3.1.2. Identification and quantification of saponins

Figure 1D displays the HPLC-UV chromatogram of saponins of hydroalcoholic extract of licorice root. A peak was found at 254 nm with the retention time of 31.37 min. It was confirmed to be glycyrrhizin by matching the retention time and spectral characteristics with that of glycyrrhizin standard at different concentrations (Figure 1C). Concentration of glycyrrhizin was calculated through a calibration graph and found to be 0.02 mg/g.

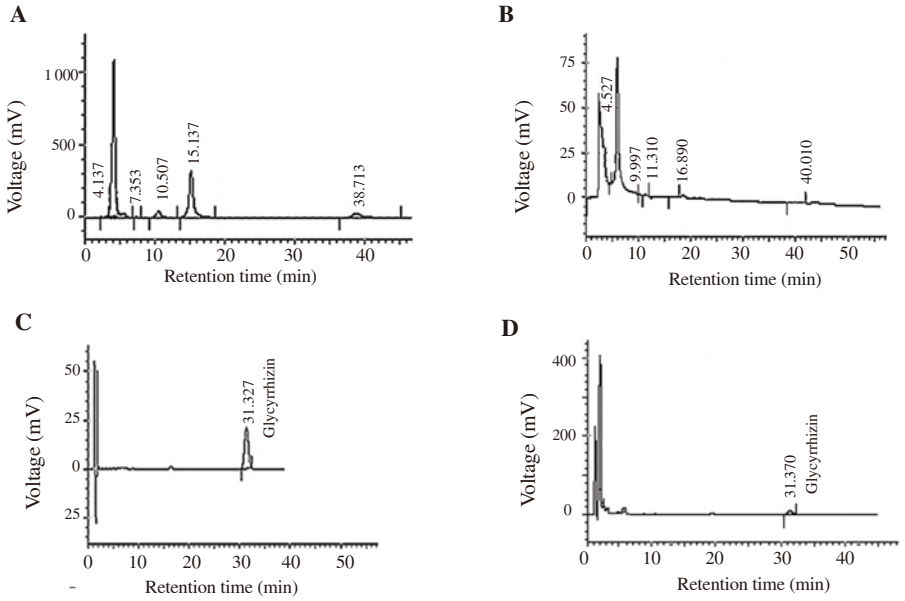

Figure 1. HPLC chromatogram of (A) flavanoid standards, RT 4.137 =rutin; RT 10.507=luteolin; RT 15.137=thymoquinone; RT 38.713=galangin; (B) flavonoids detected in hydroalcoholic extract of licorice root, RT 4.527=rutin; RT 11.310=luteolin; RT 16.890=thymoquinone; RT 40.010=galangin; (C) saponin standard, glycyrrhizin; (D) saponins detected in hydroalcoholic extract of licorice root. RT: retention time in min.

Table 1. Phytochemicals identified in hydroalcoholic extract of licorice root by GC-MS.

RT (min)	Name of the compound	Molecular formula*	Molecular weight* (g/mol)	Nature of the compound*	Bioactivity†
9.10	3-Hydroxy-3',4',5,7-tetramethoxyflavone	$C_{19}H_{18}O_7$	358.3	Flavone	Protector against lipid peroxidation, antiviral, and anti-proliferative
9.70	4H-1-Benzopyran-4-one,5,7-bis(acetyloxy)-2-[4-(acetyloxy)-3-methoxyphenyl]-3,6-dimethoxy-(jaceidin triacetate)	$C_{24}H_{22}O_{11}$	486.4	Flavone	Antibacterial
10.37	Undecane/ hendecane	$C_{11}H_{24}$	156.3	Alkane hydrocarbon	-

*PubChem, Human Metabolome Database; †science.gov, Dr. Duke's Phytochemical and Ethnobotanical Database. RT: retention time.

3.2. Phytochemical screening by GC–MS

Three compounds were detected in hydroalcoholic extract of licorice root by GC-MS analysis: 3-hydroxy-3',4',5,7-tetramethoxyflavone, jaceidin triacetate and undecane (Table 1). The GC-MS chromatogram of hydroalcoholic extract of licorice root was shown in supplementary Figure 1.

3.3. Effect of hydroalcoholic extract of licorice root on pancreatitis marker levels

Table 2 shows the activity levels of serum lipase and serum amylase in all experimental groups. Rats administered ethanol and cerulein showed significant elevation in the activity when compared to the control ($P < 0.05$). Serum levels of these markers reverted towards normal in rats treated with hydroalcoholic extract of licorice root ($P < 0.05$). Rats fed normal diet as well as hydroalcoholic extract of licorice root alone showed normal enzyme activities.

3.4. Effect of hydroalcoholic extract of licorice root on antioxidant parameters

Activities of GSH, GPx, SOD, CAT in all the groups are presented in Table 3. Their activities decreased significantly in pancreatitis-induced rats compared to the control. No significant changes were observed in the rats treated with hydroalcoholic extract of licorice root alone in comparison with the control. In rats administered ethanol and cerulein as well as hydroalcoholic extract of licorice root, the levels of these cellular antioxidants increased.

Table 2. Effect of hydroalcoholic extract of licorice root (HAELR) on lipase and amylase levels (IU/L).

Groups	Lipase	Amylase
Control	1.73±0.23	1 832.21±218.03
HAELR	1.69±0.18	1 714.02±222.82
EtOH + Cerulein	4.16±0.42*	3 872.47±398.47*
EtOH + Cerulein + HAELR	2.27±0.28#	2 298.78±264.30#

Data were analysed by one-way ANOVA followed by *post-hoc* Bonferroni test. Values are expressed as mean ± SD of six rats in each group. EtOH: ethanol. Statistical significance was calculated by comparing Control *vs.* EtOH + Cerulein; EtOH + Cerulein *vs.* EtOH + Cerulein +HAELR; *P=0.00 and #P<0.05.

3.5. Effect of hydroalcoholic extract of licorice root on lipid peroxidation

Levels of lipid peroxides including TBARS (MDA) and 4-HNE are presented in Table 4. An increase in the levels of lipid peroxidation products was found in the group administered ethanol and cerulein compared to the control. Treatment with hydroalcoholic extract of licorice root markedly reduced the levels of TBARS and 4-HNE. Levels of TBARS and 4-HNE showed no significant change in the rats treated with hydroalcoholic extract of licorice root alone.

3.6. Effect of hydroalcoholic extract of licorice root on inflammatory markers

The levels of MPO, caspase-1, IL-1 β and IL-18 in the experimental rats are presented in Table 5. A significant increase in the levels of these inflammatory markers was observed in pancreatitis-induced rats compared to the control. These levels reduced significantly in rats co-administered hydroalcoholic extract of licorice root. There were no significant changes in the levels of inflammatory markers in the group treated with hydroalcoholic extract of licorice root alone.

3.7. Histopathological observations

Figure 2 and Figure 3 illustrate the photomicrographs of pancreas and liver from all the experimental groups, respectively. Pancreatic and liver sections from the control and the group treated with hydroalcoholic extract of licorice root alone showed normal tissue architecture without any abnormal activity (Figure 2A, Figure 2B and Figure 3A, Figure 3B). Sections of the pancreas from ethanol and cerulein administered group showed neutrophil infiltration and fibrotic changes (Figure 2C). Its liver section also showed tissue infiltration with neutrophils (Figure 3C). Tissue architecture of pancreas (Figure 2D) and liver (Figure 3D) of pancreatitis-induced rats co-administered hydroalcoholic extract of licorice root at 100 mg/kg was improved.

Table 3. Effect of hydroalcoholic extract of licorice root (HAELR) on antioxidant parameters.

Groups	GSH (mg/g protein)	GPx (nM GSH oxidized/min/mg protein)	SOD (Units/mg protein)	CAT (μM of H_2O_2 consumed/min/mg protein)
Control	56.20±6.57	80.20±10.34	13.50±1.53	105.20±13.00
HAELR	55.30±6.85	79.80±9.57	13.00±1.67	103.60±11.95
EtOH + Cerulein	21.40±3.18*	48.80±6.99*	6.90±0.96*	70.90±10.45*
EtOH + Cerulein + HAELR	44.50±6.43#	68.20±8.72#	11.20±1.40#	97.40±10.90#

Data were analysed by one-way ANOVA followed by *post-hoc* Bonferroni test. Values are expressed as mean ± SD of six rats in each group. GSH: glutathione; GPx: glutathione peroxidase; SOD: superoxide dismutase; CAT: catalase; EtOH: ethanol. Statistical significance was calculated by comparing Control *vs.* EtOH + Cerulein; EtOH + Cerulein *vs.* EtOH + Cerulein +HAELR; *P=0.00 and #P<0.05.

Table 4. Effect of hydroalcoholic extract of licorice root (HAELR) on lipid peroxidation.

Groups	TBARS (MDA) (nM/mg protein)	4-HNE (μM/g protein)
Control	29.80±3.14	1.98±0.22
HAELR	30.70±3.77	2.12±0.27
EtOH + Cerulein	51.10±5.57*	8.30±0.84*
EtOH + Cerulein + HAELR	33.90±4.17#	4.89±0.59#

Data were analysed by one-way ANOVA followed by *post–hoc* Bonferroni test. Values are expressed as mean ± SD of six rats in each group. TBARS: thiobarbituric acid-reacting substances; MDA: Malondialdehyde; 4-HNE: 4-hydroxynonenal; EtOH: ethanol. Statistical significance was calculated by comparing Control *vs.* EtOH + Cerulein; EtOH + Cerulein *vs.* EtOH + Cerulein +HAELR; *P=0.00 and #P<0.05.

4. Discussion

In the present investigation, hydroalcoholic extract of licorice root was evaluated for its ameliorating effect on pancreatic inflammation using rat model with ethanol and cerulein-induced pancreatitis. Recurrent AP induced by cerulein promotes chronic changes in the pancreas. Ethanol acts as a progression factor to trigger inflammatory and immune responses[3]. Chronic ethanol administration increases the pancreatic content of cathepsin B, a lysosomal cysteine protease that causes intrapancreatic activation of trypsinogen leading to the autodigestion of pancreas. Subsequent cell injury is mediated by the activated proteases. Ethanol lessens the recovery from AP and sensitizes pancreas to chronic injury through toxic effects of its metabolites on acinar cells, oxidative stress and promoting activation of PSCs, the major fibrogenic cells in the pancreas. The innate immune response of these cells is important in the induction of pancreatic inflammation[25].

As mentioned earlier, pancreatic acinar cells are the major sites of ethanol metabolism. Oxidative metabolism of ethanol by CYP2E1 produces toxic aldehydes that lead to increased ROS, triggering oxidative stress and activation of NF- κ B. When ROS production overwhelms the antioxidant defence, the uncontrolled ROS generation causes cellular injury and dysfunction. NF- κ B is involved in regulating the gene expression of inflammatory molecules including chemokine and cytokines like TNF- α [26]. NF- κ B was shown to be activated by cerulein in rat acinar cells making it the best animal model of experimental pancreatitis. Non-oxidative ethanol metabolism involves formation of FAEE that disrupts the acinar cell membrane.

A wide range of phytoconstituents proved their role in the modulation of inflammatory response and their potency as oxidative stress modulators. Many phytonutrients work by inhibiting the inflammatory pathways as satisfactorily as NSAIDs but with less toxicity. Therefore, the focus of this investigation is on novel natural products which may shift the treatment paradigm for CP.

G. glabra, a flavoring herb, is known for its medicinal properties in the indigenous system of Ayurvedic medicine. It is also known that compounds with antioxidant properties may exert anti-inflammatory effects. Based on the previous evidences[27,28], it is hypothesized that hydroalcoholic extract of licorice root could be pancreato-protective and is investigated *in vivo*.

GC/MS is the most robust tool for plant metabolite profiling and is adopted for identifying the metabolites in hydroalcoholic extract of licorice root. Inspection of results lead to the detection of rare flavonoids such as 3-hydroxy-3',4',5,7-tetramethoxyflavone which is reported to be an excellent protector against lipid peroxidation. The 3-OH group and the electron presenting substituents like OH/-OCH3 groups at positions 5 and 7 are important for its antioxidant activity[29]. The other rare flavonoid[30] detected was jaceidin triacetate with modest antioxidative activity[31]. These compounds might contribute to the antioxidant nature of licorice roots and could have attenuated oxidative stress in pancreas.

HPLC-UV analysis showed the presence of flavonoids like rutin, quercetin and luteolin; quinones like thymoquinone; saponins like glycyrrhizin in hydroalcoholic extract of licorice root. The presence of glycyrrhizin, a pentacyclic triterpenic saponin which is anti-inflammatory[32] was also confirmed.

Both serum lipase and amylase levels were increased in pancreatitis-induced rats. These enzyme levels were reverted towards normal in rats co-administered hydroalcoholic extract of licorice root, highlighting the pancreato-protective effect of the extract. In a similar study, Abed *et al* reported that the increase in serum amylase and lipase in pancreatitis was markedly lessened by a traditional Iranian drug, prepared from flower petals of *Echium amoenum*[33].

Mitochondrion is the organelle that generates most of the adenosine triphosphate (ATP) in mammalian cells. Oxidation of various substrates results in the formation of NADH or reduced ubiquinone, which then get oxidized through the electron transport chain of the mitochondria, to drive ATP synthesis. Generation of ATP is inevitably associated with production of ROS. ROS are highly reactive molecules, generated as metabolic by-products during ATP synthesis at major sites like complex- Ⅰ and complex- Ⅲ of electron transport chain.

Ethanol, when converted to acetaldehyde by ADH and to acetate by ALDH, a molecule of NADH is produced in each reaction, supplying more reducing equivalents (NADH) to mitochondria. These excess reducing equivalents facilitate transfer of electrons to molecular oxygen propagating the cycle of excess ROS generation. Shalbueva *et al* demonstrated that ethanol treatment led to a decrease in the NAD^+/NADH ratio in isolated acinar cells[34]. So ethanol increases redox pressure in mitochondria through NADH, promoting formation of ROS. The pathologic effect of cerulein in stimulating mitochondrial ROS production was reported to be by abnormal calcium signalling[6]. Once produced, ROS can cause potential damage to cellular constituents. Under physiological conditions, ROS are safely neutralized by the antioxidant defence systems both inside and outside the mitochondria.

In pancreatitis, infiltrating inflammatory cells, ethanol metabolism through CYP2E1, NADPH oxidase of PSCs and xanthine oxidase released by damaged pancreas could act as sources of intracellular ROS production[35–37]. Detoxifying enzymes (SOD, CAT, GPx) and non-enzymatic free radical scavengers (GSH) work together in cells against free radicals to preserve ideal redox status in living cells. When the ROS levels overcome the cellular antioxidant defence as in pancreatitis, the balance shifts towards ROS formation as noted in rats administered ethanol and cerulein. After treatment

Table 5. Effect of hydroalcoholic extract of licorice root (HAELR) on inflammatory markers.

Groups	MPO (Units/mg protein)	Caspase-1		IL-1 β (pg/mL)	IL-18 (pg/mL)
		Serum (pg/mL)	Pancreas (pM/mg protein)		
Control	2.93±0.33	12.30±1.76	12.50±1.41	14.90±1.65	190.30±30.03
HAELR	2.77±0.32	11.40±1.20	11.30±1.38	14.10±1.78	201.20±25.50
EtOH + Cerulein	5.04±0.69*	23.20±2.52*	48.80±5.27*	29.70±4.12*	345.50±42.10*
EtOH + Cerulein + HAELR	3.49±0.52#	15.10±1.89#	21.20±2.54#	19.30±2.10#	219.00±29.78#*

Data were analysed by one-way ANOVA followed by *post-hoc* Bonferroni test. Values are expressed as mean±SD of six rats in each group. MPO: myeloperoxidase; EtOH: ethanol. Statistical significance was calculated by comparing Control *vs.* EtOH + Cerulein; EtOH + Cerulein *vs.* EtOH + Cerulein + HAELR; *P=0.00 and #P<0.05.

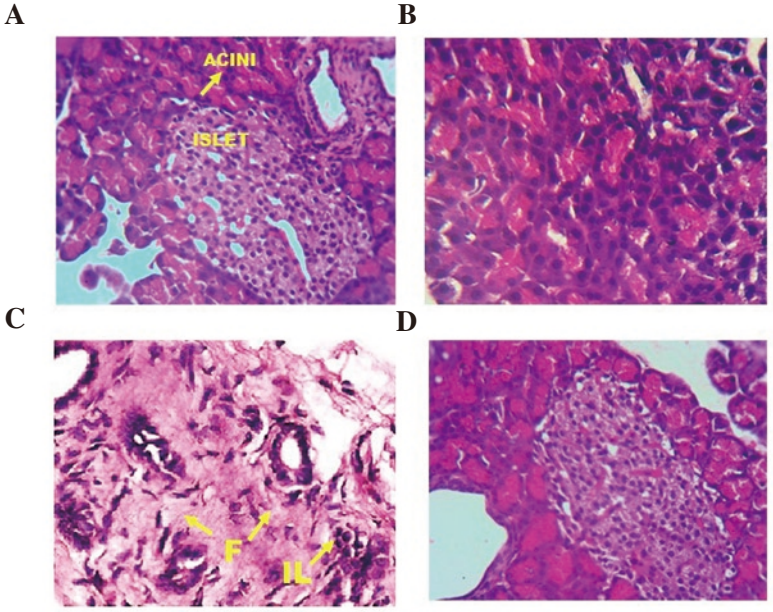

Figure 2. Histology of pancreatic tissue sections (H&E stain, 400×). (A)&(B) pancreas sections from the control rats and the rats treated with hydroalcoholic extract of licorice root alone show pristine tissue architecture; (C) sections from rats administered ethanol and cerulein show inflammatory cell infiltration (IL) and fibrosis (F); (D) sections from rats co-administered hydroalcoholic extract of licorice root show improved tissue architecture.

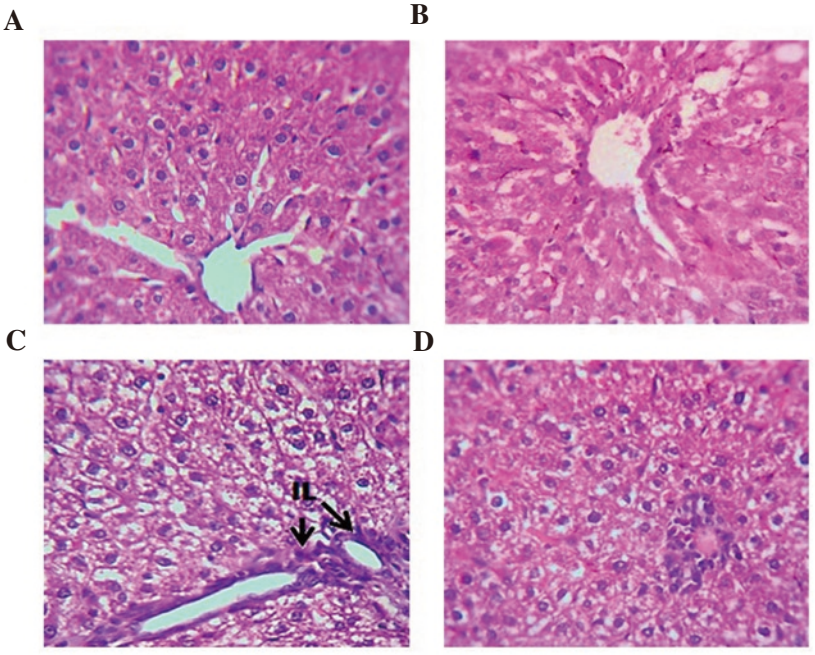

Figure 3. Histology of liver sections (H&E stain, 400×). (A)&(B) liver sections from the control rats and the rats treated with hydroalcoholic extract of licorice root alone show undisturbed structure; (C) liver tissues from rats administered ethanol and cerulein show inflammatory cell infiltration (IL); (D) liver sections from rats co-administered hydroalcoholic extract of licorice root show decreased infiltration of inflammatory cells.

with hydroalcoholic extract of licorice root, the enzyme levels were reverted towards normal, marking amelioration of oxidative stress.

Mitochondria, involved in the generation of ROS are the major targets of oxidative stress. Chronic ethanol treatment causes loss of mitochondrial GSH and inactivation of GPx and respiratory complexes. When these complexes are impaired, ROS can accumulate to toxic levels, leading to mitochondrial oxidative stress and dysfunction. Enhanced mitochondrial ROS production also induces NLRP3 inflammasome activation and associated inflammatory responses[38]. These aspects pertaining to mitochondria are focused separately in the other phase of our investigation.

Because of high chemical reactivity, ROS cause lipid peroxidation and the end products of lipid peroxidation like MDA and 4-HNE reflect the ROS-dependent tissue damage[39]. An increase in the levels of TBARS (MDA) and 4-HNE was observed in the pancreas of rats administered ethanol and cerulein, and these levels decreased significantly in rats co-administered hydroalcoholic extract of licorice root. These findings showed that treatment with hydroalcoholic extract of licorice root improved the antioxidant status and decreased the incidence of free radical-induced cell injury in pancreas.

Degree of polymorphonuclear leukocyte infiltration in the pancreas was measured in terms of tissue concentrations of MPO produced by these cells[40]. Elevation in MPO concentration was observed in the pancreas of rats received ethanol and cerulein, reflecting the tissue inflammation. MPO concentration decreased significantly in hydroalcoholic extract of licorice root treated rats, indicating its high anti-inflammatory potency. Bioactive compounds like flavonoids and saponins in hydroalcoholic extract of licorice root might contribute to its anti-inflammatory property.

ROS act as a molecular trigger of various inflammatory processes. A study by Yu *et al* has reported that cerulein generates ROS and thereby increases activation of NF- κ B, and cytokine expression suggesting a possible relationship between ROS generation and the production of inflammatory cytokines in the pancreatic acinar cells. They further highlighted that these alterations are inhibited by endogenous antioxidants[41]. ROS can even act as a chemoattractant for inflammatory cells[6]. Caspase-1, IL-1 β and IL-18 have important roles in mediating pancreatic injury and inflammation and their levels in serum significantly elevated after chronic ethanol exposure[42]. Persistent activation of PSCs by these cytokines propels pancreatic fibrosis. Existing studies revealed that, enhanced levels of IL-1 β and IL-18 induce CP[43,44]. The serum levels of caspase-1, IL-1 β and IL-18 in rats received ethanol and cerulein were higher compared to those of control rats, and decreased after hydroalcoholic extract of licorice root treatment, revealing its ability to inhibit the production of active IL-1 β and IL-18. Similar inactivation was shown to reduce the severity of pancreatitis[45]. These observations draw special attention to highly potent anti-inflammatory nature of hydroalcoholic extract of licorice root. Histopathological observations showed that hydroalcoholic extract of licorice root co-administration ameliorated the pathological alterations in tissue architecture in both pancreas and liver, underlining its protective effect.

The results of this study clearly indicate that, hydroalcoholic extract of licorice root exhibits pancreato-protective activity probably by preventing the inflammatory cascade of cytokine activation and cellular injury. This anti-inflammatory effect of hydroalcoholic extract of licorice root as observed in this study might be due to its antioxidant phytoconstituents, since ethanol and cerulein-induced ROS production contributed to the severe inflammation in pancreas. Though many medicinal properties have been proved for licorice roots, this study confirms the anti-inflammatory property which protects pancreas from ethanol mediated injury.

Conflict of interest statement

The authors declare that they have no conflict of interest.

Acknowledgments

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References

[1] Manohar M, Verma AK, Venkateshaiah SU, Sanders NL, Mishra A. Pathogenic mechanisms of pancreatitis. *World J Gastrointest Pharmacol Ther* 2017; **8**(1): 10.

[2] Machicado JD, Yadav D. Epidemiology of recurrent acute and chronic pancreatitis: Similarities and differences. *Dig Dis Sci* 2017; **62**(7): 1683-1691.

[3] Deng X, Wang L, Elm MS, Gabazadeh D, Diorio GJ, Eagon PK, et al. Chronic alcohol consumption accelerates fibrosis in response to cerulein-induced pancreatitis in rats. *Am J Pathol* 2005; **166**(1): 93-106.

[4] Gukovskaya AS, Gukovsky I, Algül H, Habtezion A. Autophagy, inflammation, and immune dysfunction in the pathogenesis of pancreatitis. *Gastroenterology* 2017; **153**(5): 1212-1226.

[5] Apte MV, Pirola RC, Wilson JS. Alcohol and the pancreas. *Pancreapedia: Exocrine pancreas knowledge base*. American Pancreatic Association; 2016. Doi: 10.3998/panc.2016.17.

[6] Kim H. Cerulein pancreatitis: Oxidative stress, inflammation, and apoptosis. *Gut Liver* 2008; **2**(2): 74.

[7] Robles L, Vaziri ND, Ichii H. Role of oxidative stress in the pathogenesis of pancreatitis: Effect of antioxidant therapy. *Pancreat Disord Ther* 2013; **3**(1): 112.

[8] Mews P, Phillips P, Fahmy R, Korsten M, Pirola R, Wilson J, et al. Pancreatic stellate cells respond to inflammatory cytokines: Potential role in chronic pancreatitis. *Gut* 2002; **50**(4): 535-541.

[9] Gukovskaya AS, Hosseini S, Satoh A, Cheng JH, Nam KJ, Gukovsky I, et al. Ethanol differentially regulates NF- κ B activation in pancreatic acinar cells through calcium and protein kinase C pathways. *Am J Physiol Gastrointest Liver Physiol* 2004; **286**(2): 204-213.

[10]Watanabe T, Kudo M, Strober W. Immunopathogenesis of pancreatitis. *Mucosal Immunol* 2017; **10**(2): 283.

[11]Xiang H, Zhang Q, Qi B, Tao X, Xia S, Song H, et al. Chinese herbal

medicines attenuate acute pancreatitis: Pharmacological activities and mechanisms. *Front Pharmacol* 2017; **8**: 216.

[12]Montero L, Ibáñez E, Russo M, di Sanzo R, Rastrelli L, Piccinelli AL, et al. Metabolite profiling of licorice (*Glycyrrhiza glabra*) from different locations using comprehensive two-dimensional liquid chromatography coupled to diode array and tandem mass spectrometry detection. *Anal Chim Acta* 2016; **913**: 145-159.

[13]Tewari D, Mocan A, Parvanov ED, Sah AN, Nabavi SM, Huminiecki L, et al. Ethnopharmacological approaches for therapy of jaundice: Part Ⅱ. Highly used plant species from Acanthaceae, Euphorbiaceae, Asteraceae, Combretaceae, and Fabaceae families. *Front Pharmacol* 2017; **8**: 519.

[14]Handa SS. An overview of extraction techniques for medicinal and aromatic plants. In: Handa SS, Khanuja SPS, Longo G, Rakesh DD (eds.) *Extraction technologies for medicinal and aromatic plants.* United nation industrial development organization and international center for Science and High Technology, Trieste, Italy; 2008, pp. 21-52.

[15]Hurst WJ, McKim JM, Martin Jr RA. High-performance liquid chromatographic determination of glycyrrhizin in licorice products. *J Agric Food Chem* 1983; **31**(2): 387-389.

[16]Lowry RR, Tinsley IJ. Rapid colorimetric determination of free fatty acids. *J Am Oil Chem Soc* 1976; **53**(7): 470-472.

[17]Gomori G. Assay of serum amylase with small amounts of serum. *Am J Clin Pathol* 1957; **27**: 714-715.

[18]Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 1976; **72**(1-2): 248-254.

[19]Moron MS, Depierre JW, Mannervik B. Levels of glutathione, glutathione reductase and glutathione S-transferase activities in rat lung and liver. *Biochim Biophys Acta* 1979; **582**(1): 67-78.

[20]Flohe L, Gunzler WA. Assays of glutathione peroxidase. *Methods Enzymol* 1984; **105**: 114-120

[21]Kakkar P, Das B, Viswanathan PN. A modified spectrophotometric assay of superoxide dismutase. *Indian J Biochem Biophys* 1984; **21**(2): 130-132.

[22]Aebi H. Catalase *in vitro*. *Methods Enzymol* 1984; **105**: 121-126

[23]Draper HH, Hadley M. Malondialdehyde determination as index of lipid peroxidation. *Methods Enzymol* 1990; **86**: 421-431.

[24]Bradley PP, Priebat DA, Christensen RD, Rothstein G. Measurement of cutaneous inflammation: Estimation of neutrophil content with an enzyme marker. *J Invest Dermatol* 1982; **78**(3): 206-209.

[25]Clemens DL, Schneider KJ, Arkfeld CK, Grode JR, Wells MA, Singh S. Alcoholic pancreatitis: New insights into the pathogenesis and treatment. *World J Gastrointest Pathophysiol* 2016; **7**(1): 48.

[26]Liu T, Zhang L, Joo D, Sun SC. NF-κB signaling in inflammation. *Signal Transduct Target Ther* 2017; **2**: 17023.

[27]Yu JY, Ha JY, Kim KM, Jung YS, Jung JC, Oh S. Anti-inflammatory activities of licorice extract and its active compounds, glycyrrhizic acid, liquiritin and liquiritigenin, in BV2 cells and mice liver. *Molecules* 2015; **20**(7): 13041-13054.

[28]Khattab HA, Abdel-Dayem UA, Jambi HA, Abbas AT, Abdul-Jawad MT, El-Shitany NA. Licorice (*Glycyrrhizza glabra*) extract prevents production of Th2 cytokines and free radicals induced by ova albumin in mice. *Int J Pharmacol* 2018; **14**(8): 1072-1079.

[29]Li YL, Guo H, Zhao YQ, Li AF, Ren YQ, Zhang JW. Quercetin protects neuronal cells from oxidative stress and cognitive degradation induced by amyloid β-peptide treatment. *Mol Med Rep* 2017; **16**(2): 1573-1577.

[30]Dhananjayan K. Molecular docking study characterization of rare flavonoids at the nac-binding site of the first bromodomain of BRD4 (BRD4 BD1). *J Cancer Res* 2015; **2015**: 1-15.

[31]Aljancić I, Stanković M, Tesević V, Vujisić L, Vajs V, Milosavljević S. Protective effect on human lymphocytes of some flavonoids isolated from two *Achillea* species. *Nat Prod Commun* 2010; **5**(5): 729-732.

[32]Wang XR, Hao HG, Chu L. Glycyrrhizin inhibits LPS-induced inflammatory mediator production in endometrial epithelial cells. *Microb Pathog* 2017; **109**: 110-113.

[33]Abed A, Minaiyan M, Ghannadi A, Mahzouni P, Babavalian MR. Effect of *Echium amoenum* Fisch. et Mey a traditional Iranian herbal remedy in an experimental model of acute pancreatitis. *ISRN Gastroenterol* 2012; **2012**: 141548

[34]Shalbueva N, Mareninova OA, Gerloff A, Yuan J, Waldron RT, Pandol SJ, et al. Effects of oxidative alcohol metabolism on the mitochondrial permeability transition pore and necrosis in a mouse model of alcoholic pancreatitis. *Gastroenterology* 2013; **144**(2): 437-446.

[35]Kikuta K, Masamune A, Satoh M, Suzuki N, Shimosegawa T. 4-hydroxy-2, 3-nonenal activates activator protein-1 and mitogen-activated protein kinases in rat pancreatic stellate cells. *World J Gastroenterol* 2004; **10**(16): 2344.

[36]Masamune A, Watanabe T, Kikuta K, Satoh K, Shimosegawa T. NADPH oxidase plays a crucial role in the activation of pancreatic stellate cells. *Am J Physiol Gastrointest Liver Physiol* 2008; **294**(1): G99-108.

[37]Folch E, Gelpi E, Rosello-Catafau J, Closa D. Free radicals generated by xanthine oxidase mediate pancreatitis-associated organ failure. *Dig Dis Sci* 1998; **43**(11): 2405-2410.

[38]Liu Q, Zhang D, Hu D, Zhou X, Zhou Y. The role of mitochondria in NLRP3 inflammasome activation. *Mol Immunol* 2018; **103**: 115-124.

[39]Kwiecien S, Jasnos K, Magierowski M, Sliwowski Z, Pajdo R, Brzozowski B, et al. Lipid peroxidation, reactive oxygen species and antioxidative factors in the pathogenesis of gastric mucosal lesions and mechanism of protection against oxidative stress-induced gastric injury. *J Physiol Pharmacol* 2014; **65**(5): 613-622.

[40]Simsek O, Kocael A, Kocael P, Orhan A, Cengiz M, Balcı H, et al. Inflammatory mediators in the diagnosis and treatment of acute pancreatitis: Pentraxin-3, procalcitonin and myeloperoxidase. *Arch Med Sci* 2018; **14**(2): 288.

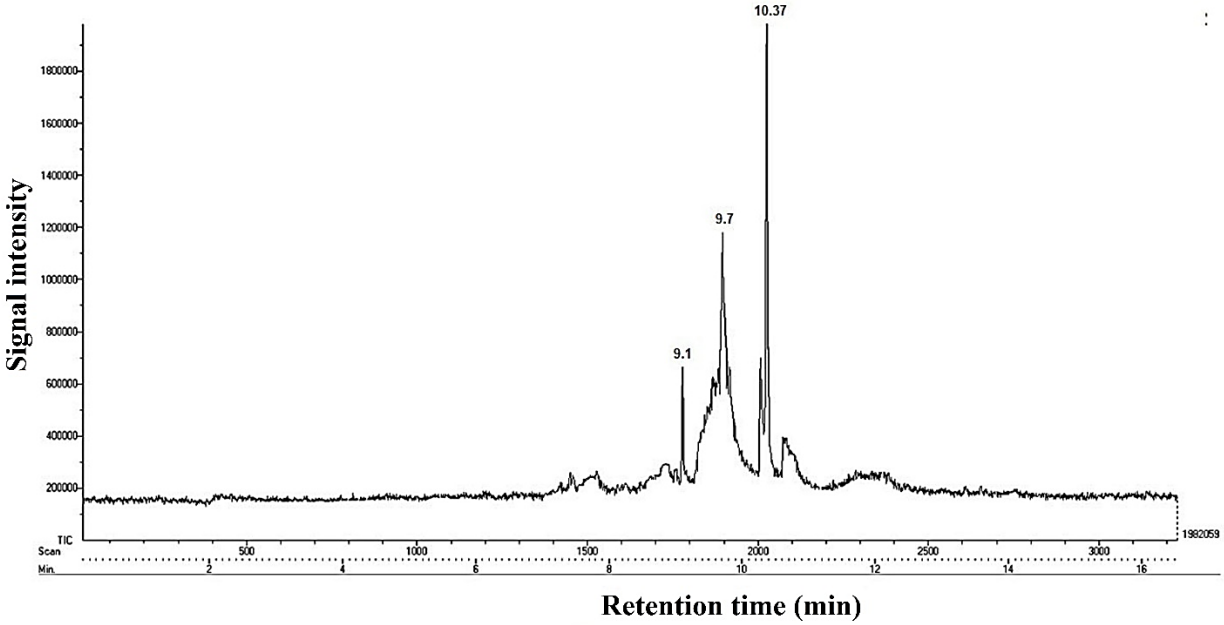
[41]Yu JH, Lim JW, Namkung W, Kim H, Kim KH. Suppression of cerulein-induced cytokine expression by antioxidants in pancreatic acinar cells. *Lab Invest* 2002; **82**(10): 1359.

[42]Hoque R, Mehal WZ. Inflammasomes in pancreatic physiology and disease. *Am J Physiol Gastrointest Liver Physiol* 2015; **308**(8): G643-651.

[43]Marrache F, Tu SP, Bhagat G, Pendyala S, Österreicher CH, Gordon S, et al. Overexpression of interleukin-1 β in the murine pancreas results in chronic pancreatitis. *Gastroenterology* 2008; **135**(4): 1277-1287.

[44]Schneider A, Haas SL, Hildenbrand R, Siegmund S, Reinhard I, Nakovics H, et al. Enhanced expression of interleukin-18 in serum and pancreas of patients with chronic pancreatitis. *World J Gastroenterol* 2006; **12**(40): 6507.

[45]Ning JW, Zhang Y, Yu MS, Gu ML, Xu J, Usman A, et al. Emodin alleviates intestinal mucosal injury in rats with severe acute pancreatitis *via* the caspase-1 inhibition. *Hepatobil Pancreat Dis Int* 2017; **16**(4): 431-436.



Supplementary Figure 1. Total ion chromatogram from the GC-MS analysis of hydroalcoholic extract of licorice root. RT 9.1= 3-Hydroxy-3',4',5,7-tetramethoxyflavone; RT 9.7= jaceidin triacetate; RT 10.37= undecane. RT: retention time in min.