

Full Length Research Paper

The effect of *Flos Lonicerae* on caerulein-induced pancreatitis in rats

Jong-Kil Jeong¹ and Jeong-Sang Kim^{2*}

¹Department of Herbology, College of Oriental Medicine, Dongshin University, Naju, Republic of Korea.

²Department of Anatomy, College of Oriental Medicine, Dongshin University, Naju, Republic of Korea.

Accepted 10 October, 2011

Acute pancreatitis (AP) is associated with significant morbidity and mortality; however, there is no specific treatment for this disease. Our objective is to study how *Flos Lonicerae*, an herbal medicine used for treating inflammation within oriental medicine practices, affects inflammation and acinar cell injury during the early phase of AP. A total of twenty-four male Sprague-Dawley rats were randomly allocated into a normal group, a control group, and two AP with *F. Lonicerae* extract groups. A single dose of *F. Lonicerae* water extract (100 or 200 mg/kg) was co-administered with caerulein 2 h before AP induction. Plasma amylase activities, the concentration of platelet activating factor (PAF), tumor necrosis factor (TNF- α), and interleukin 6 (IL-6) were measured. Pancreatic sections were histological and COX-2 immunohistologically assessed for abnormal acinar cells and interstitial space. AP induction produced a sevenfold increase in plasma amylase and a threefold increase in interstitial space. Observations also showed that 90% of the acinar cells were abnormal. For rats from the AP *F. Lonicerae* extract groups, serum amylase and IL-6 were reduced by 86 and 84% relative to the control group ($p < 0.05$) at 6 h, respectively. Histological severity of pancreatitis, graded on the basis of pancreatic edema, acinar cell vacuolization and inflammation, was reduced in the *F. Lonicerae* extract administered rats. These results suggest that *F. Lonicerae* water extract has an ameliorative effect for suppressing inflammation on caerulein-induced AP in rat.

Key words: *Flos Lonicerae*, pancreatitis, immunohistochemistry.

INTRODUCTION

Acute pancreatitis (AP) is an inflammatory disease of the pancreas as a result of significant morbidity and mortality (Pandol et al., 2007). Various cause, including gallstones, alcohol, trauma, infections and genetic alterations, have been implicated in the causation of this disease (Andrew and O'Reilly, 2006; Pandol, 2006). It is characterized by intra-acinar cell activation of digestive enzymes, pancreatic inflammation, cell damage and a systemic inflammatory response governed by the release of

proinflammatory cytokines (Pandol, 2006). AP has been the subject of investigations for many years. In addition to the hypotheses of premature trypsinogen activation, changes in the cytoskeleton, the colocalization hypothesis and the disruption of exocrine protein transport, cytokines have become a new focus in the investigation of the pathophysiology of pancreatitis (Bhatia et al., 2001).

Here, TNF- α and IL-6 as proinflammatory cytokines play a predominant role (Bhatia and Mochhala, 2004). In previous reports, chlorogenic acid, one of the major components in *F. Lonicerae*, has been widely adopted to control the quality of *F. Lonicerae* owing to its high content and antibiotic property (Chai et al., 2005; Song et al., 2006; Chen et al., 2007). To date, few reports exist on the role of the *F. Lonicerae* in regulating exocrine pancreatic secretion. We studied whether the administration of

*Corresponding author. E-mail: jskim@dsu.ac.kr.

Abbreviations: AP, Acute pancreatitis; PAF, platelet activating factor; TNF- α , tumor necrosis factor; IL-6, interleukin 6; DAB, 3,3-diaminobenzidine tetrahydrochloride; SPSS, statistically package for social sciences; mRNA, messenger ribonucleic acid.

herbal medicine, *F. lonicerae* has long been known for its anti-inflammatory and cancer chemopreventive activities and is used in the traditional Chinese medicine (Joo et al., 2010), was able to reduce inflammation in an experimental model of caerulein-induced AP in rats. Hyperstimulating doses of caerulein (50 µg/kg) administered in five injections per mouse given at hourly intervals produced the following: high serum amylase activity; increases in TNF-α and IL-6; and pancreatic injury evaluated by histological analysis. There is a growing interest in natural compounds for the treatment of various inflammatory disorders. In this study, we tested whether *F. lonicerae* is effective for *in vivo* application in caerulein-induced pancreatitis. We found that *F. lonicerae* inhibited the inflammatory response and significantly ameliorated pancreatitis in both models.

MATERIALS AND METHODS

Animals and experimental design

Male Sprague dawley rats (210±10 g) were obtained from the (Samtaco Company Korea). The experimental procedures were approved by the Animal Experimentation Ethics Committee of Dongshin University of Korea. The animals were housed in temperature-controlled (23 ± 2°C) rooms with a 12 h light-dark cycle. The rats had free access to water and all rats were fed a standard laboratory diet until an overnight fast before the experiment. Rats were allocated to the following groups (n = 6): normal (Nor), control (Con), and *F. lonicerae* water extracts administration (100 or 200 mg/kg).

Administration of herbal extract and induction of pancreatitis

Rats were given caerulein (50 µg/kg body wt) by intraperitoneal injection at hourly intervals for a total of five injections and killed at 6 h. Some animals were pretreated with 100 or 200 mg/kg of *F. lonicerae* (*Lonicerae japonicae*) extract 2 h before the start of caerulein administration.

Measurement of pancreatic edema

Pancreas was obtained 6 h after the first caerulein injection to weigh a wet tissue weight. The Pancreatic weight ratios were calculated as the wet pancreas weight/wet body weight. Pancreatic edema was expressed in terms of pancreatic-to-body mass (Chan and Leung, 2006).

Serum amylase concentration

Mixed arteriovenous blood was centrifuged for 10 min at 1,500 g. The serum amylase concentration was measured with a Fuji DRI-CHEM (FDC 4000i, FUJI, Japan) as instructed by the manufacturer, and serum amylase was expressed as milligrams per milliliter.

Determination of platelet-activating factor (PAF)

PAF levels were determined using a commercially available PAF acetylhydrolase assay kit (Cayman, USA). Each sample was

measured in duplicate with a microplate reader (SpectraMax M2, Molecular Device, USA).

Determination of TNF-α and IL-6 levels

TNF-α and IL-6 levels were measured using a commercially available ELISA kit for rat TNF-α and IL-6 (BD, USA) according to the manufacturer's protocol. Each sample was measured in duplicate with a microplate reader (SpectraMax M2, Molecular Device, USA).

Histology

Portions of the pancreas were fixed overnight at room temperature in a pH-neutral, phosphate-buffered, 10% formalin solution. The tissue was then embedded in paraffin, sectioned at 5 µm, stained with hematoxylin and eosin and examined with a light microscope connected to a digital camera (Coolpix 4500, Nikon, Tokyo, Japan).

Immunohistochemistry

Sections of 5 µm were cut from one representative block for each case and collected on gelatin-coated slides. The slides were deparaffinized and rehydrated in an alcohol series. Endogenous peroxidase was blocked by immersion in 3% hydrogen peroxide. Deparaffinized tissue sections were subjected to heat-induced antigen retrieval (water bath at 98°C) with antigen retrieval solution (DAKO, SA, Denmark, pH 6.0). Next, the slides were incubated at 4°C for 16 h with a primary rabbit monoclonal anti-goat Cox-2 antibody (SP21, 1:20, Lab Vision, Fremont, CA) and an anti-goat Cd31 mouse monoclonal (JC70A, 1:50, DAKO), 14 followed by the EnVision polymer HRP and Envision+ (DAKO) for 1 h at 37°C. The sections were stained with the chromogen 3,3-diaminobenzidine tetrahydrochloride (DAB Substrate System, Lab Vision), incubated for 10 minutes and counterstained with Mayer's hematoxylin and examined with a light microscope connected to a digital camera (Coolpix 4500, Nikon, Tokyo, Japan).

Statistical analysis

All data are expressed as mean ± S.E. values. The statistical analyses were performed on a statistically package for social sciences (SPSS) (Chicago, IL) program. T-tests were used to the differences between groups. Student's t-test was used to compare the differences between groups. Values of $p < 0.05$ were considered significant.

RESULTS

Effect of *F. lonicerae* extract pretreatment on caerulein-induced pancreatic edema

Rats were given caerulein and killed at 6 h. The pancreatic weight ratios were calculated as the wet pancreas weight/wet body weight. Figure 1 shows intense edema in the control group compared with the normal group and two experimental groups. As shown in Figure 1, *F. lonicerae* extract pretreatment at a single dose of 200 mg/kg b.wt significantly ($^{\#}p < 0.05$) reduced the degree of edema, whereas 100 mg/kg b.wt treatment was less

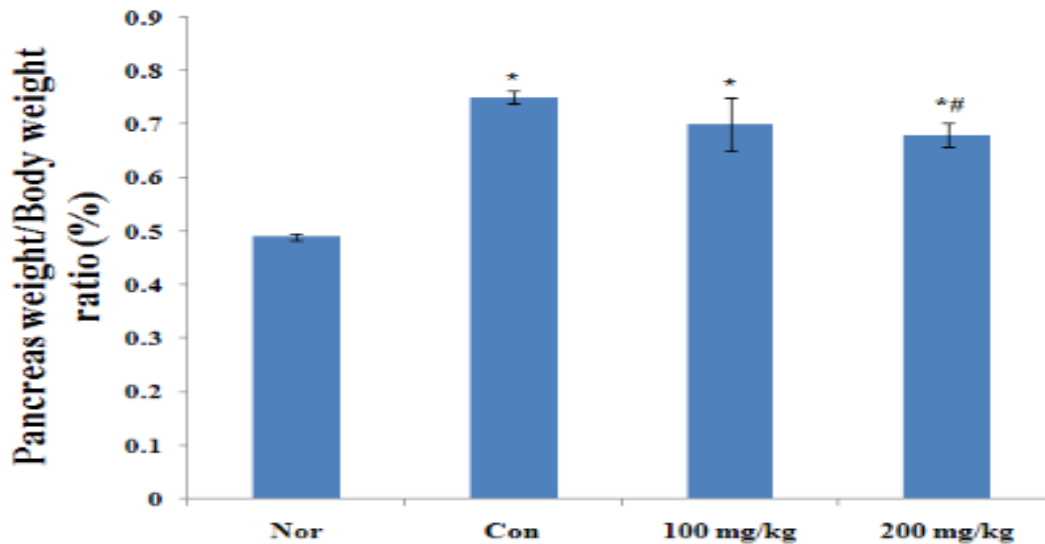


Figure 1. The comparison of pancreas/body weight. Normal group (Nor) was administrated with saline only. Control group (Con) was administrated with saline treatment with caerulein. Experimental groups were pretreated with *F. lonicerae* extract at 100 or 200 mg/kg body weight 2 h before the start of caerulein administration. All values are mean \pm S.E. (n = 6). Significant differences were compared with normal/control at * $p < 0.05$ /# $p < 0.05$.

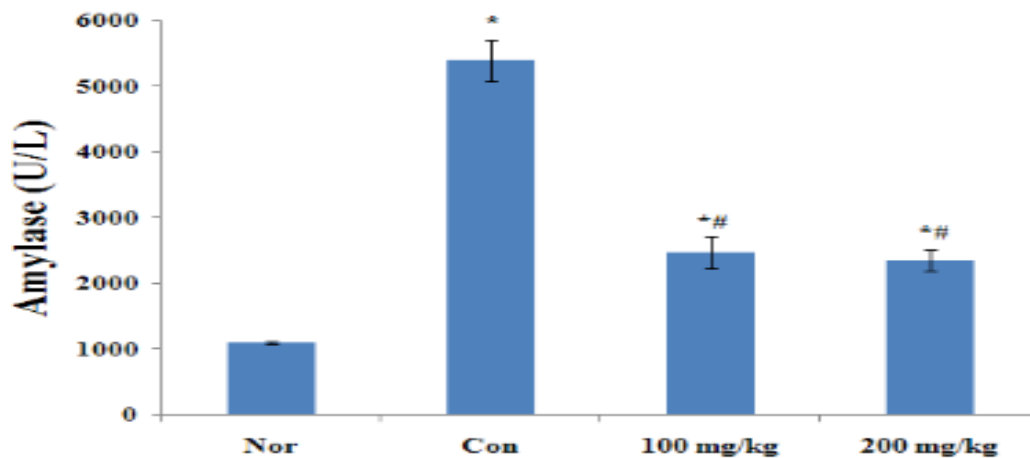


Figure 2. The changes of serum amylase activities. Nor was administrated with saline only. Con was administrated with saline treatment with caerulein. Experimental groups were pretreated with *F. lonicerae* extract at 100 or 200 mg/kg body weight 2 h before the start of caerulein administration. All values are mean \pm S.E. (n = 6). Significant differences were compared with normal/control at * $p < 0.05$ /# $p < 0.05$.

effective.

Amylase activities

The induction of AP produced about a fivefold increase in plasma amylase activity (Figure 2). *F. lonicerae* extract pretreatment with caerulein reduced plasma amylase activity by up to 236% compared with the activity in the AP-alone control group, the reduction in amylase release

by pancreatic cells was significant (* $p < 0.05$) and dose-dependent.

The changes of serum platelet activating factor (PAF) activities

The induction of AP produced about a twentyfold increase in the plasma PAF activities compared with the Nor (Figure 3). *F. lonicerae* extract pretreatment with

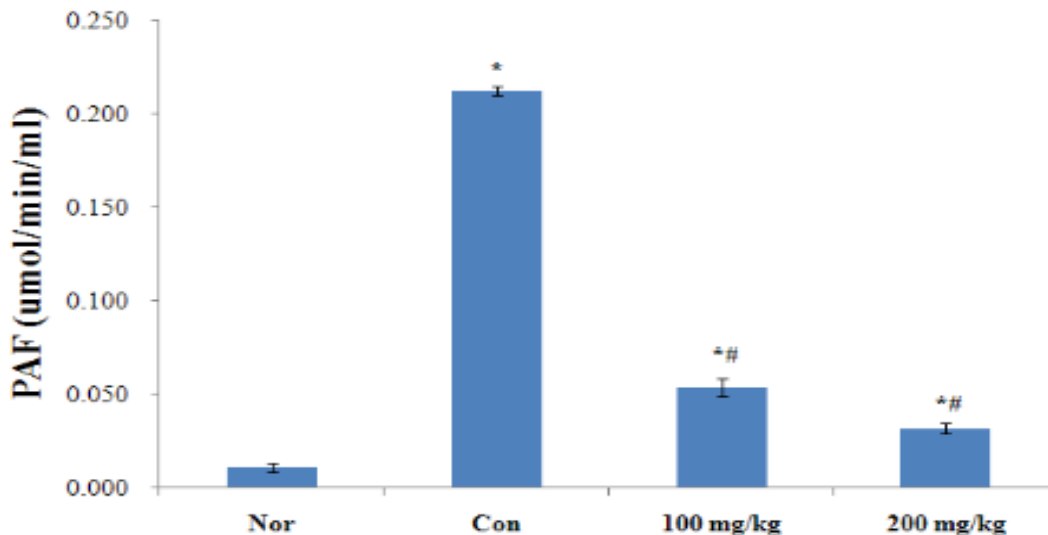


Figure 3. The changes of serum PAF activities. Nor was administrated with saline only. Con was administrated with saline treatment with caerulein. Experimental groups were pretreated with *F. lonicerae* extract at 100 or 200 mg/kg body weight 2 h before the start of caerulein administration. All values are mean \pm S.E. (n = 6). Significant differences were compared with normal/control at * $p < 0.05$ /# $p < 0.05$.

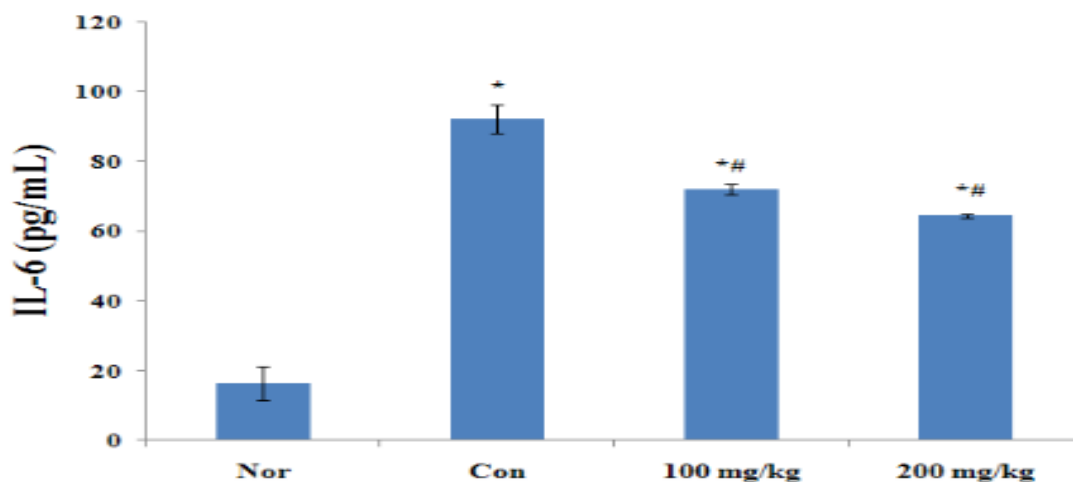


Figure 4. The changes of IL-6 concentrations. Nor was administrated with saline only. Con was administrated with saline treatment with caerulein. Experimental groups were pretreated with *F. lonicerae* extract at 100 or 200 mg/kg body weight 2 h before the start of caerulein administration. All values are mean \pm S.E. (n = 6). Significant differences were compared with normal/control at * $p < 0.05$ /# $p < 0.05$.

caerulein reduced plasma PAF activities by up to 600% compared with the activity in the AP-alone control group. The reduction in PAF activities were significant (* $p < 0.05$) and dose-dependent.

The changes of serum interleukin-6 (IL-6) concentration

IL-6 was evaluated because of their direct involvement in the prime and amplification of inflammation mechanisms. The induction of AP produced about a sixfold increase in

the plasma IL-6 concentration compared with the normal group (Figure 4). *F. lonicerae* extract pretreatment with caerulein reduced plasma IL-6 concentration by up to 31% compared with the activity in the AP-alone group and the reduction in IL-6 concentrations were significant (* $p < 0.05$) and dose-dependent.

Tumor necrosis factor (TNF- α) activities

TNF- α were evaluated because of their direct involvement in the prime and amplification of

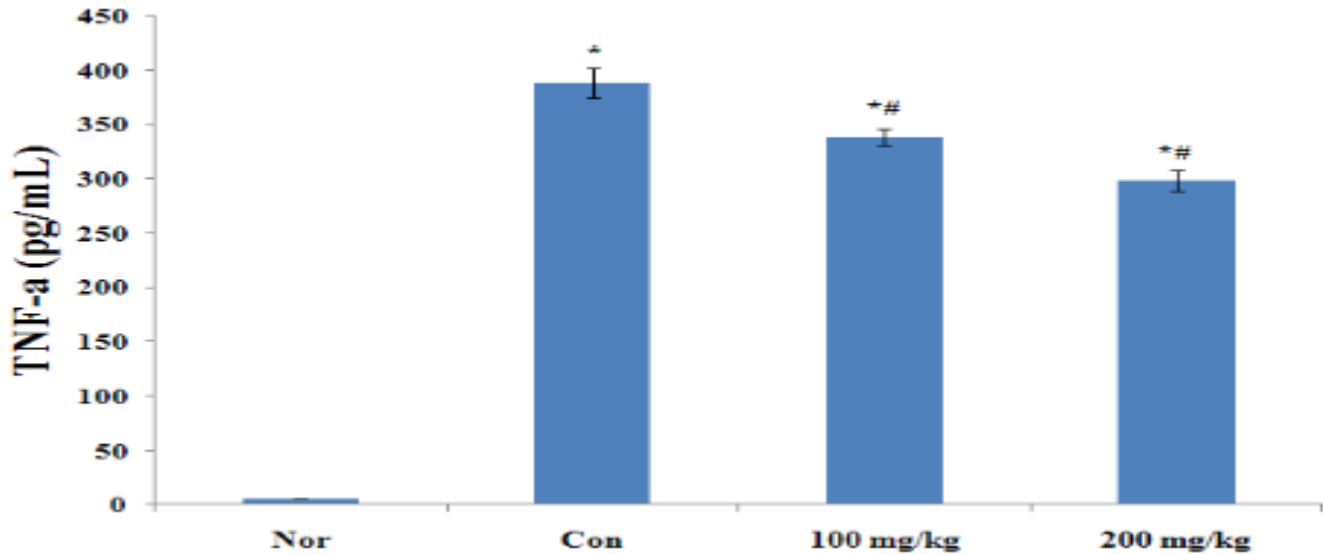


Figure 5. The changes of TNF- α activity. Nor was administrated with saline only. Con was administrated with saline treatment with caerulein. Experimental groups were pretreated with *F. Ionicerae* extract at 100 or 200 mg/kg body weight 2 h before the start of caerulein administration. All values are mean \pm S.E. (n = 6). Significant differences were compared with normal/control at *p < 0.05/#p < 0.05.

inflammation mechanisms. Figure 5 showed that the activities of these pro-inflammatory cytokines were not stimulated in caerulein-untreated normal rats. Otherwise, TNF- α activity were significantly unregulated in the animals given only caerulein of the control group. The pretreatment of *F. Ionicerae* extract was able to reduce by up to 186% compared with the activity in the AP-alone control group and the reduction in TNF- α activities were significant (#p < 0.05) and dose-dependent.

Histological studies of pancreas in caerulein-induced pancreatitis: Effect of *F. Ionicerae* extracts pretreatment

Saline-injected normal rats showed no evidence of pancreatic edema or other characteristics of AP (Figure 6a). Caerulein treatment of control rats resulted in AP characterized by increased edema, inflammation, vacuolization and necrosis. The interstitial space of pancreas increased threefold and about 90% of the acinar cells were observed as being abnormal (Figure 6b). In contrast, treatment of *F. Ionicerae* extract with caerulein led to markedly attenuated inflammatory changes that would normally be associated with AP including vacuolization, necrosis and inflammation (Figure 6c, d).

COX-2 Expression in normal pancreatic tissues

COX-2 expression was not detected in epithelial cells of normal pancreatic ducts or in normal acinar cells (Figure

7a), but COX-2 expression was strongly detected in the epithelial cells of the caerulein induced control rats (Figure 7b). COX-2 expression was slightly more decreased in the *F. Ionicerae* extract preadministered rats than control rats (Figure 7c and d).

DISCUSSION

It has been well accepted that the development of AP is a multi-step process. Whereas the onset is triggered by early acinar events, the severity and systemic inflammation is determined by consecutive extra-acinar events (Mofidi et al., 2006). The activation of trypsinogen to trypsin plays a key role in this process, leading to cell injury in experimental models of acute pancreatitis and in isolated acinar cells (Saluja et al., 1997; Li et al., 2009). The subsequent extra-acinar events consist mainly of a distinct immune response, in which proinflammatory cytokines play a major role (Bhatia et al., 2001; Mayer et al., 2000; Schmid and Adler, 1999; Simon and Sarah, 2011). Surprisingly, although amylase and lipase are important for the diagnosis of AP, neither one was included in the scores of severity. Indeed, these factors are imprecise in predicting the outcome of the disease. In AP, the normal secretory pathway of the acinar cell appears to fail. Zymogen granules are discharged into the intracellular spaces from the basolateral cell surface rather than into the duct lumen and trypsinogen is prematurely converted into trypsin. The release of activated zymogen and the presence of trypsin and other activated proteases as well as amylase and lipase contribute to acinar cell injury (Ranson, 1982).

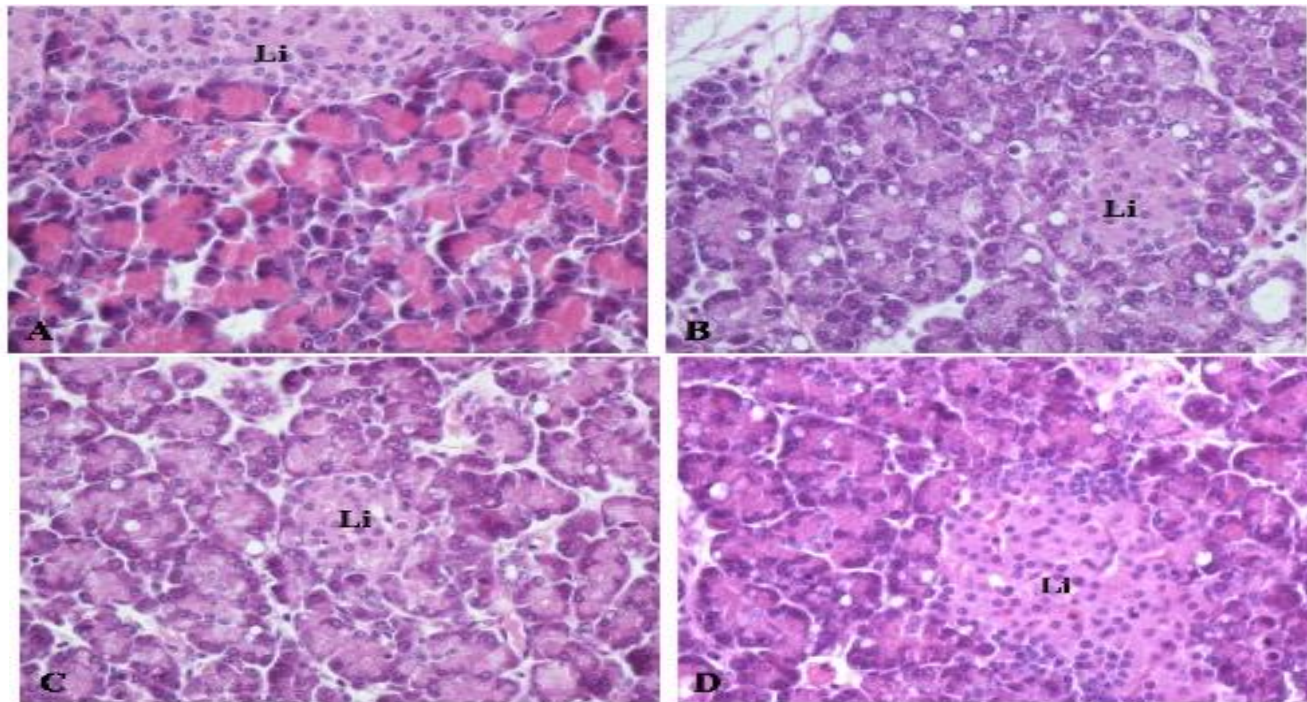


Figure 6. Light micrographs of pancreatitis at 6 h. Morphological changes in cerulein-induced pancreatitis. Representative hematoxylin-eosin-stained sections of pancreas were examined by light microscopy in normal rats; **A**, caerulein treatment control rats; **B**, *F. Ionicerae* extract 100 mg/kg preadministered; **C** and *Lonicera Flos* extract 200 mg/kg preadministered rats; **D**. Original magnification set at $\times 400$.

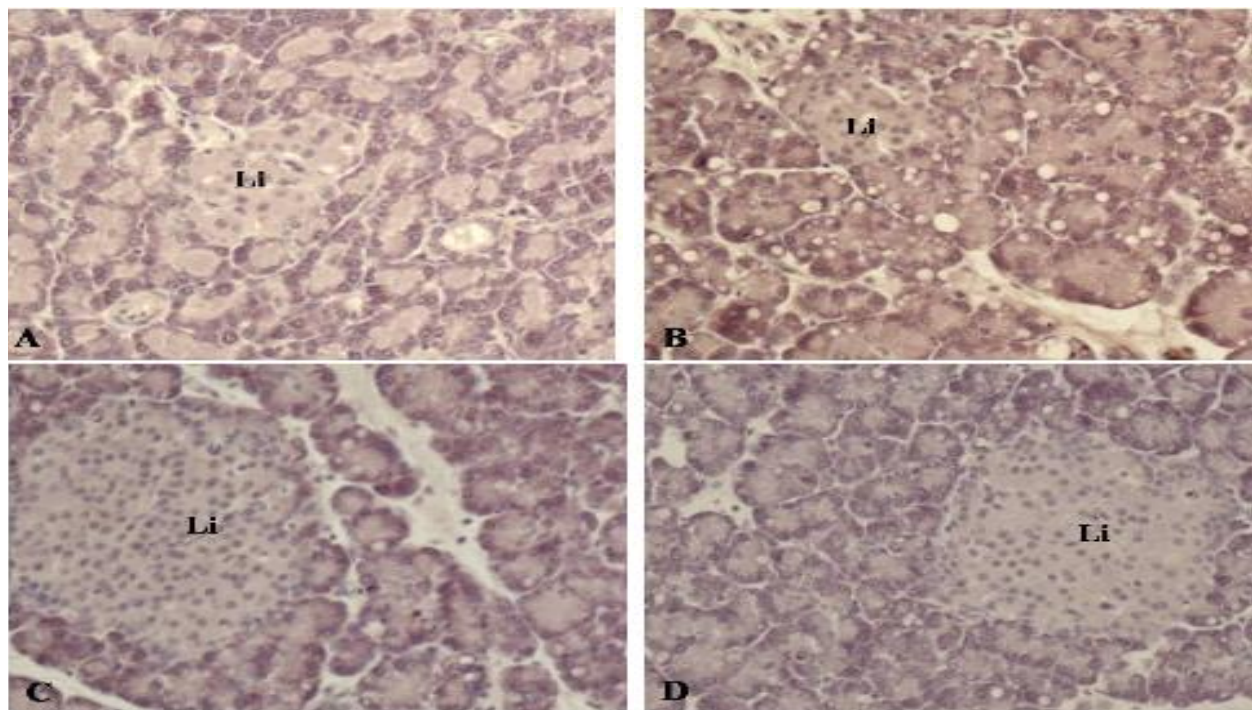


Figure 7. COX-2 immunohistochemical analysis at 6 h. Normal pancreatic tissue epithelial cells of the pancreatic duct or acinar cells are negative for COX-2; **A**, Pancreas from rat injected with caerulein alone shows extensive COX-2 expression; **B**, Pancreas from rat injected with *F. Ionicerae* extract 100 mg/kg; **C** and 200 mg/kg; **D** show weakly positive staining for COX-2 in acinar cells. Li, Langerhans islet. COX-2 immunostain, Original magnification set at $\times 400$.

F. loniceræ extract pretreatment in this study was associated with reduced hyperenzymemia.

AP is an inflammatory process which occurs in a normal organ and which is diagnosed mainly by acute abdominal pain associated with a concomitant rise of serum amylase and lipase concentrations (Steinberg and Tenner, 1994; Cheng et al., 2010). Several studies have shown that pancreatic acinar cells express chemokines and cytokines including IL-6 and TNF- α (Schafer et al., 2005). For IL-6, its role as a proinflammatory cytokine in AP so far is unclear. Suzuki et al. (2000) reported that over expressing human IL-6 in transgenic mice increased the severity of pancreatitis, suggesting it is proinflammatory. Another study suggested that IL-6 is in fact of an anti-inflammatory cytokine (Cuzzocrea et al., 2002). TNF- α is a pleiotropic cytokine with effects on cell growth, gene expression and cell death and pancreatic acini have been shown to activate, release and respond to TNF- α by apoptosis (Gukovskaya et al., 1997). The activation of pancreatic enzymes is not the only finding involved in the pathophysiology of the disease. A local inflammation is initiated which results in the local production of inflammatory mediators. Experimental studies show that pancreatic injury is mediated by the release of proinflammatory mediators such as IL-1, 6 and 8. TNF is released by macrophages within pancreatic tissue, correlates with the severity of the experimental disease (Norman et al., 1996; George et al., 2011). The present study clearly demonstrates that *F. loniceræ* extract treatment has a therapeutic effect in the protection of the development of severe acute experimental pancreatitis in rats.

The main findings of this study are as follows. First, data supported the anti-inflammatory role of *F. loniceræ* extract in acute experimental pancreatitis. It is also known that pro-inflammatory and cytotoxic cytokines play an important role in the cell injury during AP (Pereda et al., 2006). In our study, the treatment of AP in rats with *F. loniceræ* extract was able to prevent cell damage induced by caerulein treatment. Cyclooxygenase-2 (COX-2) and its products are crucially involved in various types of inflammatory responses (Park and Christman, 2006). COX-2 Messenger ribonucleic acid (mRNA) and protein levels were increased during experimental pancreatitis, but the role of COX-2 in pancreatitis has not been well defined. Moreover, accumulating evidence indicates the essential contribution of COX-2 to cerulein-induced AP, because inhibition of the COX-2 pathway also ameliorates pancreatitis (Ethridge et al., 2002). Evidence is accumulating based on the anti-inflammatory effects of inhibitors that PGE₂, a product of COX-2, is indispensable for the development of AP (Song et al., 2002). Our data show that *F. loniceræ* extract ameliorates pancreatitis in experimental models by inhibiting the inflammatory response. The results suggest a potential therapeutic role for *F. loniceræ* extract, which is currently in clinical trials for the treatment of pancreatitis. The mechanism underlying *F. loniceræ* extract's beneficial effect on AP is incompletely understood, but the present findings

provide some insight. Therapeutic administration of *F. loniceræ* extract at the dose of 100 or 200 mg/kg used in this study was not completely effective. However, this may not be the optimal dose. In summary, this study has demonstrated that *F. loniceræ* extract can ameliorate AP. This observation is of particular clinical significance because of the mortality rate attributed to severe AP.

ACKNOWLEDGEMENT

This research was supported by the Dongshin University research grants.

REFERENCES

- Andrew K, O'Reilly D (2006). Acute Pancreatitis. *B.M.J.*, 332: 1072.
- Bhatia M, Moochhala S (2004). Role of inflammatory mediators in the pathophysiology of acute respiratory distress syndrome. *J. Pathol.*, 202: 145.
- Bhatia M, Neoptolemos JP, Slavin J (2001). Inflammatory mediators as therapeutic targets in acute pancreatitis. *Curr. Opin. Investig. Drugs*, 2: 496.
- Chai XY, Li SL, Li P (2005). Quality evaluation of *Flos Loniceræ* through a simultaneous determination of seven saponins by HPLC with ELSD. *J. Chromatogr.*, 1070: 43.
- Chan YC, Leung PS (2006). AT1 receptor antagonism ameliorates acute pancreatitis-associated pulmonary injury. *Regul. Pept.*, 134: 46.
- Chen J, Song Y, Li P (2007). Capillary high-performance liquid chromatography with mass spectrometry for simultaneous determination of major flavonoids, iridoid glucosides and saponins. *J. Chromatogr.*, 1157: 217.
- Cheng S, Yan WM, Yang B, Shi JD, Song MM, Zhao Y (2010). A crucial role of nitric oxide in acute lung injury secondary to the acute necrotizing pancreatitis. *Hum. Exp. Toxicol.*, 29: 329.
- Cuzzocrea S, Mazzon E, Dugo L, Centorrino T, Ciccolo A, McDonald MC, de Sarro A, Caputi AP, Thiemermann C (2002). Absence of endogenous interleukin-6 enhances the inflammatory response during acute pancreatitis induced by cerulein in mice. *Cytokine*, 18: 274.
- Ethridge RT, Chung DH, Slogoff M, Ehlers RA, Hellmich MR, Saito SH, Uchida T, Evers BM (2002). Cyclooxygenase-2 gene disruption attenuates the severity of acute pancreatitis and pancreatitis-associated lung injury. *Gastroenterology*, 123: 1311.
- George P, Eric RW, Emily SM, Johanna ML, Jeremy SD, Michael LS (2011). TNF- α -dependent regulation of acute pancreatitis severity by Ly-6Chi monocytes in Mice. *J. Biol. Chem.*, 286: 13327.
- Gukovskaya AS, Gukovsky I, Zaninovic V, Song M, Sandoval D, Gukovsky S, Pandolf SJ (1997). Pancreatic acinar cells produce, release, and respond to tumor necrosis factor- α : Role in regulating cell death and pancreatitis. *J. Clin. Invest.*, 100: 1853.
- Joo JS, Kim JS, Jeong JG, Kim BK (2010). Study of efficacy of *Foeniculi fructus* and *Flos Loniceræ* extract on acute pancreatitis. *Korea J. Herbol.*, 25: 39.
- Li YY, Ochs S, Gao ZR, Malo A, Chen CJ, Lv S, Gallmeier E, Göke B, Schäfer C (2009). Regulation of HSP60 and the role of MK2 in a new model of severe experimental pancreatitis. *Am. J. Physiol. Gastrointest Liver. Physiol.*, 297: G981.
- Mayer J, Rau B, Gansauge F, Beger HG (2000). Inflammatory mediators in human acute pancreatitis: clinical and pathophysiological implications. *Gut*, 47: 546.
- Mofidi R, Duff MD, Wigmore SJ, Madhavan KK, Garden OJ, Parks RW (2006). Association between early systemic inflammatory response, severity of multiorgan dysfunction, and death in acute pancreatitis. *Br. J. Surg.*, 93: 738.
- Norman JG, Fink GW, Messina J, Carter G, Franz MG (1996). Timing of tumor necrosis factor antagonism is critical in determining outcome in murine lethal acute pancreatitis. *Surgery*, 120: 515.

- Pandolf SJ (2006). Acute pancreatitis. *Curr. Opin. Gastroenterol.*, 22: 481.
- Pandolf SJ, Saluja AK, Imrie CW, Banks PA (2007). Acute pancreatitis: bench to the bedside. *Gastroenterology*, 132: 1127.
- Park GY, Christman JW (2006). Involvement of cyclooxygenase-2 and prostaglandins in the molecular pathogenesis of inflammatory lung diseases. *Am. J. Physiol.*, 290: L797.
- Pereda J, Sabater L, Aparisi L, Escobar J, Sandoval J, Vina J, Lopez-Rodas G, Sastre J (2006). Interaction between cytokines and oxidative stress in acute pancreatitis. *Curr. Med. Chem.*, 13: 2775.
- Ranson JH (1982). Etiologic and prognostic factors in human acute pancreatitis: a review. *Am. J. Gastroenterol.*, 77: 633.
- Saluja AK, Donovan EA, Yamanaka K, Yamaguchi Y, Hofbauer B, Steer ML (1997). Cerulein-induced *in vitro* activation of trypsinogen in rat pancreatic acini is mediated by cathepsin B. *Gastroenterology*, 113: 304.
- Schafer C, Tietz AB, Goke B (2005). Pathophysiology of acute experimental pancreatitis: lessons from genetically engineered animal models and new molecular approaches. *Digestion*, 71: 162.
- Schmid RM, Adler G (1999). Cytokines in acute pancreatitis: new pathophysiological concepts evolve. *Eur. J. Gastroenterol. Hepatol.*, 11: 125.
- Simon JFH, Sarah CC (2011). Acute pancreatitis. *Ann. Clin. Biochem.*, 48: 23.
- Song AM, Bhagat L, Singh VP, Van Acker GG, Steer ML, Saluja AK (2002). Inhibition of cyclooxygenase-2 ameliorates the severity of pancreatitis and associated lung injury. *Am. J. Physiol.*, 283: G1166.
- Song Y, Li SL, Wu MH, Li HJ, Li P (2006). Qualitative and quantitative analysis of iridoid glycosides in the flower buds of *Lonicera* species by capillary high performance liquid chromatography coupled with mass spectrometric detector. *Anal. Chim. Acta.*, 564: 211.
- Steinberg W, Tenner S (1994). Acute pancreatitis. *N. Engl. J. Med.*, 330: 1198.
- Suzuki S, Miyasaka K, Jimi A, Funakoshi A (2000). Induction of acute pancreatitis by cerulein in human IL-6 gene transgenic mice. *Pancreas*, 21: 86.