

Effects of *Atractylodis Rhizoma* Pharmacopuncture on an Acute Gastric Mucosal Lesion Induced by Compound 48/80 in Rats

Yun-Kyu Lee, Jae-Soo Kim, Seong-Chul Lim

Department of Acupuncture & Moxibustion, Daegu Haany University College of Oriental Medicine, Daegu, Korea

Key Words

Acupuncture; Acute gastric mucosal lesion; ARP (*Atractylodis Rhizoma* pharmacopuncture); Compound 48/80; Joksamni (ST36); Jung-wan (CV12)

Abstract

Objectives: This study was designed to investigate the protective effects of *Atractylodis Rhizoma* pharmacopuncture (ARP) against acute gastric mucosal lesions induced by compound 48/80 in rats.

Methods: The ARP was injected in Joksamni (ST36) and Jung-wan (CV12) 1 hr before treatment with compound 48/80. The animals were sacrificed under anesthesia 3 hrs after treatment with compound 48/80. The stomachs were removed, and the amounts of gastric adherent mucus, gastric mucosal hexosamine, thiobarbituric acid reactive substances (TBARS), xanthine oxidase (XO), and superoxide dismutase (SOD) were measured. Also, histological examination were performed.

Results: Gastric adherent mucus, gastric mucosal hexosamine and histological defects of gastric mucosa declined significantly after ARP treatment. Changes in gastric mucosal TBARS were also reduced by ARP treatment, but this result was not statistically significant. ARP treatment did not change the XO and the SOD activities.

Conclusions: ARP showed protective effects for acute gastric mucosal lesions induced by compound 48/80 in rats. These results suggest that ARP may have protective effects for gastritis.

Received: Jan 18, 2012 Accepted: Mar 19, 2012

1. Introduction

Gastritis appears to result from reproduction of gastric epithelial cells after the gastric mucositis, which is a common disease among digestive diseases [1]. In medicine, there are many drugs for gastritis [2]. Recently developed drugs for gastritis are mainly H₂ receptor blockers, because histamine plays an important role in the production of stomach acid. In Korean medicine, *Atractylodis Rhizoma*, roots of *Atractylodes lancea* DC. and *Atractylodes chinensis* Koidzumi, is used to strengthen the spleen, and treat stomach disorders [3].

Many gastritis animal models exist, and the H₂ receptor blocking effect via compound 48/80 can be evaluated using the acute gastritis model. Compound 48/80 is known to cause degranulation of connective tissue mast cells, but not mucosal mast cells, with the release of serotonin and histamine from the cells [4,5].

The reactive oxygen species (ROS) is toxic oxygen free radicals that cause the oxidative damage involved in cell injury and inflammation [6,7]. Gastric mucin is known to interact with ROS [8].

So far few reports about the effects of *Atractylodis Rhizoma* and *Atractylodis Rhizoma* pharmacopuncture (ARP) on gastritis have been reported [9-12]. However, no reports on the protective effects of ARP for acute gastritis, such as the latest research trends for acute gastritis induced by compound 48/80, to the best of our knowledge, exist. Then, in the present study, we investigated the protective effects of ARP against acute gastric mucosal lesions induced by compound 48/80. Gastric mucosal thiobarbituric acid reactive substances (TBARS, an index of lipid peroxidation), xanthine oxidase (XO) and superoxide dismutase (SOD, an enzyme to scavenge O₂ to H₂O₂ and O₂⁻) were measured for investigating

the antioxidative activity as well.

2. Materials and methods

2.1. Preparation of ARP

Atractylodis Rhizoma was purchased from Omniherb (Daegu, Korea). ARP was prepared as follows: One-hundred gram (100 g) of Atractylodis Rhizoma in 2,000 ml of distilled water was heated in a heating extractor for 3 hrs. The extracts were filtered and concentrated by using a rotary evaporator. The extracts were lyophilized by using a freeze dryer (14.2%). The lyophilized extracts were dissolved in water and filtered three times through microfilter paper (Whatman no. 2, 0.45 - 0.2 μ m). The extracts were then placed in disinfected vials, which were sealed for further study.

2.2. Reagents

Sodium chloride was purchased from Duksan (Ansan, Gyeonggi-do, Korea). Saccharose and perchloric acid were purchased from Daejung (Siheung, Gyeonggi-do, Korea). Alcian blue was purchased from BDH Laboratory Supplies (Poole, UK). Sodium phosphate monobasic and sodium phosphate dibasic were purchased from Jin Chemical (Tokyo, Japan). All other reagents were purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.3. Experimental animals

Male Wistar rats, aged six weeks (weights: about 225 - 235 g), were purchased from Samtaco Co. (Osan, Gyeonggi-do, Korea). The animals were housed in cages in a ventilated animal room with controlled temperature ($23 \pm 2^\circ$ C) and relative humidity ($55 \pm 5\%$) and with 12 hrs of light (7:00 to 19:00). The animals had free access to rat chow (Samtaco Co., Osan, Gyeonggi-do, Korea) and tap water ad libitum for one week. All animals received humane care in compliance with the guidelines of the Animal Care and Use Committee.

2.4. Gastric mucosal lesion induced by compound 48/80

For the experiment, the rats were divided into 4 groups: normal group, control group, positive control group and experimental group. The number of rats in each group was eight. All rats were 7 weeks old, had fasted for 24 hrs, and had free access to water, but not food, during the experiment.

The normal group and the positive control group were respectively administrated distilled water and famotidine (4 mg/kg) orally. The control group and the experimental group were injected with distilled water and ARP, respectively. The injected acupoints were Joksamni (ST36) on the left and the right legs and Jung-wan (CV12) on the abdomen. The anatomical locations of the stimulated acupoints in the rats were determined in accordance with other reports [13,14]. For the injections, 26 gauge safti syringes (Becton Dickinson Korea, Inc., Seoul, Korea) were inserted into a depth of 2 - 3 mm.

Compound 48/80 (0.75 mg/kg body weight), dissolved in distilled water, was intraperitoneally injected into the control group, the positive control group and the experimental group. In these groups, acute gastric mucosal lesions were induced within 1 hr of the above treatment. The normal group received an intraperitoneal injection of an equal volume of distilled water.

The rats were sacrificed under ether anesthesia within 3 hrs of compound 48/80 injection. The stomachs were removed, inflated with 10 ml of 0.9% NaCl, and put into 10% formalin for 10 mins. The stomachs were then opened along the greater curvature.

2.5. Determinations of gastric adherent mucus and gastric mucosal hexosamine, TBARS, XO, SOD

For the assays of gastric adherent mucus and gastric mucosal hexosamine, TBARS, XO, SOD, gastric mucosal tissues were homogenized in 9 volumes of ice-cold 0.05 M Tris-HCl buffer (pH 7.4). The homogenate was centrifuged at 4° C (10,000 x g, 20 mins), and the resultant supernatant was dialyzed against 100 volumes of the same buffer at 4° C for 24 hrs. Gastric mucosal SOD was assayed using the manufacturer's protocol (Dojindo, Kumamoto, Japan). XO activity was assessed by measuring the increase in absorbance at 292 nm following the formation of uric acid at 30° C. One unit of this enzyme is defined as the amount of enzyme forming 1 μ m of uric acid per min (manufacturer's protocol, Cayman chemical, Michigan, USA). Gastric mucosal TBARS was spectrophotometrically determined by using the manufacturer's protocol (Zeptomatrix, New York, USA). Hexosamine obtained from the hydrolyzed mucin was assayed using acetylacetone and Ehrlich's reagent. Gastric adherent mucus was assayed by using the method of Kitagawa et al. [15] as follows: The removed stomach was cut open along the greater curvature and rinsed with 10 mL of ice-cold 0.25 M sucrose. Then, 50 mm² (approx. 8 mm in diameter) of the glandular portion of the stomach was excised with a scalpel, and the excised part was weighed. The excised stomach was soaked in 2 mL of 0.1% alcian blue, which was dissolved in 0.16 M sucrose buffered with 0.05 M sodium acetate (pH 5.8) for 2 hrs. Uncomplexed dye was removed by using two successive washes in 2 ml of 0.25M sucrose for 15 and 45 mins, respectively then, the dye complex with mucus was extracted with 30% DSS for 2 hrs. After centrifugation (3,000 rpm for 10 mins), the optical density of the solution of alcian blue extracted with DSS was read at 620 nm, and the concentration of the extracted alcian blue was calculated in comparison with a calibration curve obtained with alcian blue solutions of known concentrations. The concentration of gastric mucosal adherent mucus is expressed as that of alcian blue adhered to the gastric mucosal surface (μ g/g tissue).

2.6. Histological examination

Samples of the corpus were excised, transformed to 10% fresh formalin and later processed by using routine techniques before embedding in paraffin. Sections (2 mm thickness) were mounted on glass slides and stained with successive stainings using alcian blue, hematoxylin, and eosin. Coded slides were examined with a light microscope (BX60, Olympus, Tokyo, Japan) by an experienced pathologist blinded to the treatment.

2.7. Statistical analysis

The results were expressed as means \pm standard errors of the mean (SEM). Significances of changes were evaluated using the one-way ANOVA and Dunnett's post-hoc test (SPSS ver. 10.0). Values of $p < 0.05$ were considered significant.

3. Results

3.1. Effect of ARP on the gastric adherent mucus content

Gastric adherent mucus in the control group was reduced to $38.2 \pm 0.9\%$. Both the positive control and the ARP groups were significantly protected against the loss of mucus (adherent mucus: $68.5 \pm 12.3\%$ and $68.5 \pm 7.2\%$, respectively) (Fig. 1).

3.2. Effect of ARP on the gastric mucosal hexosamine content

Gastric mucosal hexosamine content showed patterns similar to those of gastric adherent mucus. Gastric mucosal hexosami-

ne content in the control group was reduced to $45.2 \pm 6.2\%$. Both the positive control and the ARP groups were significantly protected against the loss of mucus (mucosal hexosamine: $73.6 \pm 11.9\%$ and $77.9 \pm 9.6\%$, respectively) (Fig. 2).

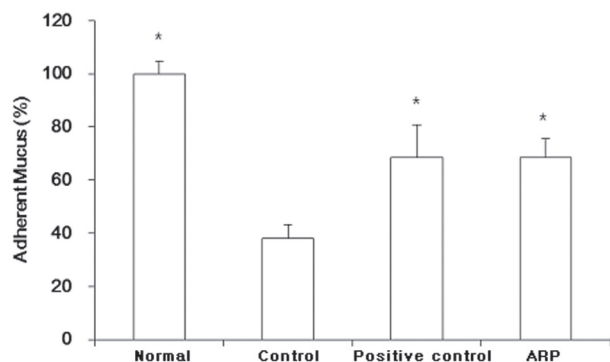


Figure 1 Effect of ARP on the gastric adherent mucus content in rats with and without a compound 48/80 injection. Normal: Rats ($n = 8$) were injected with distilled water. Control: Rats ($n = 8$) were injected with compound 48/80 and distilled water acupuncture. Positive control: Rats ($n = 8$) were injected with compound 48/80 and administrated famotidine. ARP: Rats ($n = 8$) were injected with compound 48/80 and ARP. * significantly different from the control, $p < 0.05$.

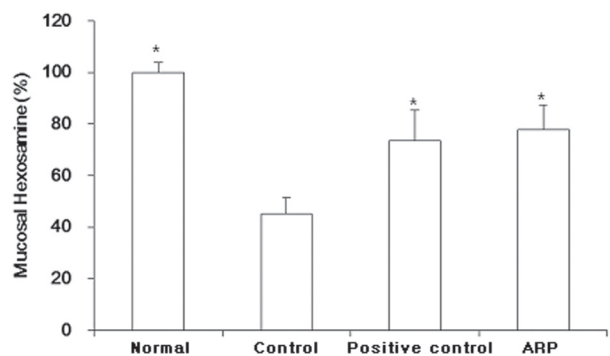


Figure 2 Effect of ARP on the gastric mucosal hexosamine content in rats with and without a compound 48/80 injection. Normal: Rats ($n = 8$) were injected with distilled water. Control: Rats ($n = 8$) were injected with compound 48/80 and distilled water acupuncture. Positive control: Rats ($n = 8$) were injected with compound 48/80 and administrated famotidine. ARP: Rats ($n = 8$) were injected with compound 48/80 and ARP. * significantly different from the control, $p < 0.05$.

3.3. Effect of ARP on the gastric mucosal lesion

As shown in Fig. 3, gastric mucosal lesions appeared 0.5 hr after treatment with compound 48/80 (Fig. 3B). Alcian blue stained the mucosal layer to dark blue (upper layer in Fig. 3). The mucosal layer was thinned after compound 48/80 treatment. Both famotidine and ARP treatments protected the mucosal layer (Figs. 3C and D).

3.4. Effect of ARP on the gastric mucosal TBARS content

TBARS in the normal group were $1.3 \pm 0.5 \mu\text{M}/\text{protein}$. In comparison with the normal group, TBARS in the control group

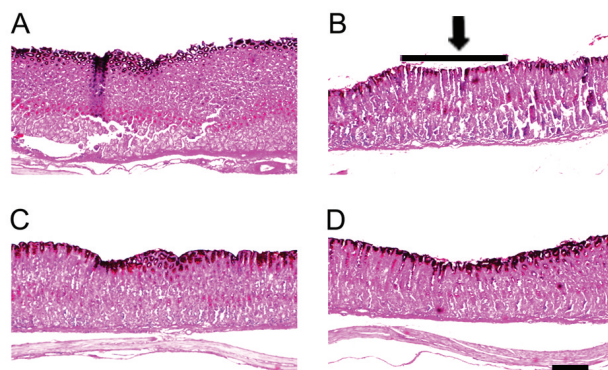


Figure 3 Photomicrographs of gastric mucosal tissues. A: Rats ($n = 8$) were injected with distilled water. B: Rats ($n = 8$) were injected with compound 48/80 and distilled water acupuncture. C: Rats ($n = 8$) were injected with compound 48/80 and administrated famotidine. D: Rats ($n = 8$) were injected with compound 48/80 and ARP. Scale bar located in figure D means $200 \mu\text{m}$. Darkly stained region is the mucosal layer of gastric tissue. Arrow region means the gastric mucosal lesion.

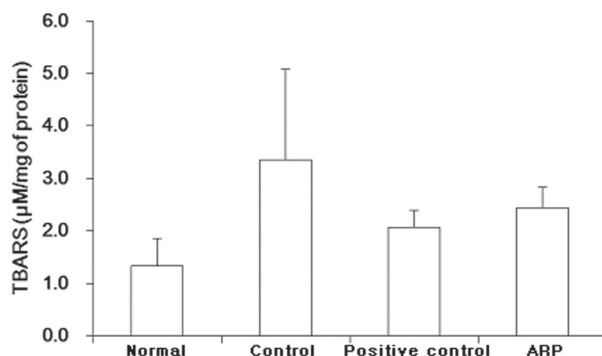


Figure 4 Effect of ARP on the gastric mucosal TBARS content in rats with and without a compound 48/80 injection. Normal: Rats ($n = 8$) were injected with distilled water. Control: Rats ($n = 8$) were injected with compound 48/80 and distilled water acupuncture. Positive control: Rats ($n = 8$) were injected with compound 48/80 and administrated famotidine. ARP: Rats ($n = 8$) were injected with compound 48/80 and ARP.

were increased, to $3.4 \pm 1.7 \mu\text{M}/\text{protein}$. In the positive control and the ARP groups the TBARS contents were $2.1 \pm 0.3 \mu\text{M}/\text{protein}$ and $2.4 \pm 0.4 \mu\text{M}/\text{protein}$, respectively (Fig. 4). However, none of the changes showed any statistical significance.

3.5. Effect of ARP on the gastric mucosal XO activity

XO in the normal group was $2288.1 \pm 1105.5 \mu\text{U}/\text{protein}$. In comparison with the normal group, XO in control group was slightly lower $1769.5 \pm 399.4 \mu\text{U}/\text{protein}$. The positive control group showed XO at $1369.9 \pm 143.8 \mu\text{U}/\text{protein}$, and the ARP group showed XO at $2056.6 \pm 356.6 \mu\text{U}/\text{protein}$ (Fig. 5).

3.6. Effect of ARP on the gastric mucosal SOD activity

SOD in the normal group was $585.7 \pm 213.9 \text{U}/\text{protein}$. SOD in

the control group was higher, 820.9 ± 209.4 U/protein. The positive control and the ARP groups showed SOD activities of $667.4 \pm 62.2\%$ and $694.3 \pm 73.7\%$, respectively (Fig. 6).

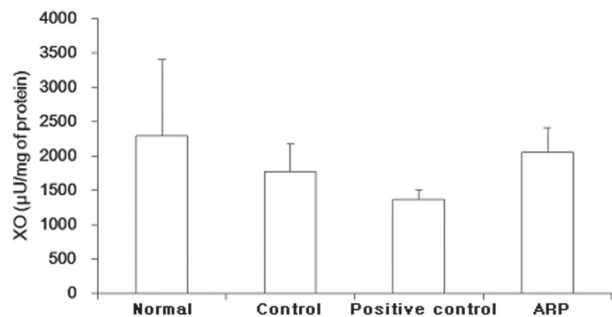


Figure 5 Effect of ARP on the gastric mucosal XO activity in rats with and without a compound 48/80 injection.

Normal: Rats ($n = 8$) were injected with distilled water.

Control: Rats ($n = 8$) were injected with compound 48/80 and distilled water acupuncture.

Positive control: Rats ($n = 8$) were injected with compound 48/80 and administrated famotidine.

ARP: Rats ($n = 8$) were injected with compound 48/80 and ARP.

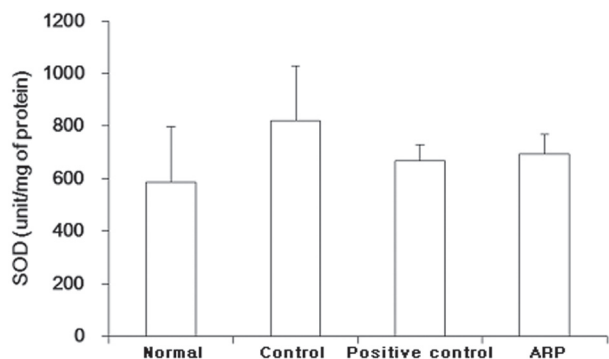


Figure 6 Effect of ARP on the gastric mucosal SOD activity in rats with and without a compound 48/80 injection.

Normal: Rats ($n = 8$) were injected with distilled water.

Control: Rats ($n = 8$) were injected with compound 48/80 and distilled water acupuncture.

Positive control: Rats ($n = 8$) were injected with compound 48/80 and administrated famotidine.

ARP: Rats ($n = 8$) were injected with compound 48/80 and ARP.

4. Discussion

Gastritis should be confirmed based on a diagnosis of inflammatory cell in fibrating the gastric mucosa, but can be identified by flares, edema and ulceration on the gastric mucosa accompanying heartburn, epigastric pain, nausea and vomiting [1].

Experimental studies have investigated the use of *Atractylodis Rhizoma* for protecting the gastric mucosa and supporting the intestinal immune system, and for its anti-inflammatory effect [9-11]. Treatments used *Atractylodis Rhizoma* also have effects on mast-cell-mediated inflammatory responses. These effects proceed from a mechanism for regulating mast cells through histamine and H_2 receptors [16]. Accordingly, ARP might be thought to have H_2 blocking effects. In this study, famotidine (H_2 receptor blockers) was used as a positive control for comparison with ARP [17].

So far, most reports have studied the use of orally-administered *Atractylodis Rhizoma* for protection of the gastric mucosa and for anti-inflammation in cases of gastritis [9-11]. Kim et al. [12] reported the effects of ARP on acute gastritis. However, some differences, method and acupoints, exist between both Kim et al.'s [12] report and this one. In this study, compound 48/80 was used to induce acute gastric mucosal lesions. Many studies revealed that a single treatment of compound 48/80 in rats could cause gastric mucosal lesions to occur [15,18,19]. ARP was injected at Joksamni (ST36) and Jung-wan (CV12) in this study. Both ST36 and CV12 are recognized as the most important acupoints for stomach disorders. ST36 is simultaneously the acupoint of the Stomach meridians, which has an excellent effect on stomach disorders, and the main acupoint of all diseases. CV12, which is the acupoint of the Conception vessel, can be utilized in the treatment of gastric disease due to the relation with Stomach meridians [20].

In the present study, ARP could significantly protect against the loss of gastric adherent mucus. Gastric adherent mucus in control group was $38.2 \pm 0.9\%$. Both the positive control and the ARP groups were significantly protected against the loss of mucus ($68.5 \pm 12.3\%$ and $68.5 \pm 7.2\%$, respectively). The effect was almost equal to that for the positive control drug, famotidine (Fig. 1). ARP could also significantly protect against the loss of gastric mucosal hexosamine. The gastric mucosal hexosamine content in the control group was $45.2 \pm 6.2\%$. Both the positive control and the ARP groups were significantly protected against the loss of mucus ($73.6 \pm 11.9\%$ and $77.9 \pm 9.6\%$, respectively).

Increasing the gastric mucosal hexosamine contributes to both the prevention and the healing of gastric mucosal lesions [21]. The protection against the loss of gastric mucosal hexosamine was almost equal to that for the positive control drug, famotidine (Fig. 2). The mucosal lesions caused by the compound 48/80 showed thin mucosal layers (darkly-stained region of gastric tissue) in photomicrographs. ARP and famotidine produced almost the same protection of the mucosal layer as was observed in the normal group (Fig. 3).

Gastric mucin is well known to interact with ROS [22].

ROS is toxic oxygen free radicals that cause the oxidative damage involved in cell injury and inflammation [6,7]. Gastric mucin is known to interact with ROS, especially the hydroxyl radical in vitro [8]. The accumulation of ROS can modulate many processes in the gastric epithelium [23].

Concentrations of TBARS are an index of lipid peroxidation and oxidative stress, TBARS being increased by oxidation [24]. In this study, TBARS contents were higher in the control group and were lower in both the famotidine- and ARP-treated groups. TBARS contents in the control group were $3.4 \pm 1.7 \mu\text{M}/\text{protein}$ compared to $1.3 \pm 0.5 \mu\text{M}/\text{protein}$ in the normal group. Both the positive control (famotidine-treated) and the experimental (ARP-treated) groups had lower TBARS contents ($2.1 \pm 0.3 \mu\text{M}/\text{protein}$ and $2.4 \pm 0.4 \mu\text{M}/\text{protein}$, respectively) (Fig. 4). Both famotidine and ARP treatments are thought to affect the antioxidative process, but this result had no statistical significance.

XO has an increasing tendency for ROS-oxidation [25,26]. In this study, XO in the control group was slightly decreased to $1769.5 \pm 399.4 \mu\text{U}/\text{protein}$ from $2288.1 \pm 1105.5 \mu\text{U}/\text{protein}$ (normal group). The positive control group showed $1369.9 \pm 143.8 \mu\text{U}/\text{protein}$, and the experimental (ARP-treated) group showed $2056.6 \pm 356.6 \mu\text{U}/\text{protein}$. Compound 48/80 treatment on the contrary slightly decreased the XO activity, but famotidine and ARP treatments did not change the activity much (Fig. 5).

SOD has an increasing tendency for neutralizing ROS that generate oxidation, there forming TBARS in human organism

[7,24]. In this study, SOD in the control group was increased to 820.9 ± 209.4 U/protein. The normal group showed 585.7 ± 213.9 U/protein. Both the positive control and the experimental (ARP-treated) groups showed lower levels of SOD activities ($667.4 \pm 62.2\%$ and $694.3 \pm 73.7\%$, respectively). SOD on the contrary was increased after compound 48/80 treatment. Both famotidine and ARP treatments reduced the elevation of SOD activities (Fig. 6).

In conclusion, ARP showed protective effects of the gastric mucosa against acute gastritis induced by compound 48/80. Although the famotidine treatment group were oral administration as contrasted with the ARP treatment group, which were injection, ARP has coordinative effects on H₂ receptor blockers in comparison with famotidine. These results suggest that ARP may have potential as an anti-gastric mucosal lesion treatment. Further studies investigating the mechanisms, more effective acupoints, and differences in ARP concentrations are needed.

5. Conclusion

The *Atractylodis Rhizoma* pharmacopuncture (ARP) was injected at Joksamni (ST36) and Jung-wan (CV12) 1 hr before compound 48/80 treatment. The amounts of gastric adherent mucus, gastric mucosal hexosamine, TBARS, XO, and SOD were measured, and histological examinations were performed. The results were as follow,.

1. ARP could significantly protect against the loss of gastric adherent mucus.
2. ARP could significantly protect against the loss of gastric mucosal hexosamine content.
3. ARP could protect the mucosal layer on histological examination.
4. ARP reduced the elevation of TBARS content, but this result had no statistical significance.
5. ARP did not change the XO and the SOD activities.

The results showed that ARP had protective effects against acute gastric mucosal lesions induced by compound 48/80 in rats. Further studies investigating the mechanisms, more effective acupoints, and differences in ARP concentrations are needed.

References

1. Lee EL, Feldman M. Gastritis and other gastropathies. In: Feldman M, Friedman LS, Sleigenger MH, eds. Sleigenger & Fordtran's gastrointestinal and liver disease, 7th ed. Philadelphia: WB Saunders Company, 2002:810-27.
2. Cho SY, Lee SH, Choi JY, Shin EM, Kang SS, Jeong SJ, et al. Antigastric effect of carbenoxolone disodium from glycyrrhizae radix. *J Toxicol Pub Health*. 2007;23(2):165-72.
3. Kim IR, Kim HC, Kuk YB, Park SJ, Park YK, Park JH, et al. Boncho-Hak. Seoul: Young-Lim Press, 2007:331-2.
4. Enerbäck L, Lundin PM. Ultrastructure of mucosal mast cells in normal and compound 48-80-treated rats. *Cell Tissue Res*. 1974;150(1):95-105.
5. Irman-Florjanc T, Erjavec F. Compound 48/80 and substance P-induced release of histamine and serotonin from rat peritoneal mast cells. *Agents Actions*. 1983;13(2-3):138-41.
6. Lee HL, Kim YS, Park JH, Chung WY, Lee KS, Oh YJ, et al. Selectively decreased expression of peroxiredoxins induced by silica in pulmonary epithelial cells. *Korean J Int Med*. 2009;24(3):220-6.
7. Kim JY. Antioxidants in sepsis. *Korean J Crit Care Med*. 2010;25(2):57-60.
8. Grisham MB, Von Ritter C, Smith BF, Lamont JT, Granger DN. Interaction between oxygen radicals and gastric mucin. *Am J Physiol. Gastrointestinal and Liver Physiology*. 1987;253(1):G93-6.
9. Yu KW, Shin KS. Identification of Pectic Polysaccharide Possessing Bone Marrow Cell Proliferation Activity through Peyer's Patch Cells from rhizomes of *Atractylodes lancea* DC. *Food Sci Biotechnol*. 2001;10(4):437-45.
10. Li CQ, He LC, Jin JQ. Atractylenolide I and atractylenolide III inhibit lipopolysaccharide-induced TNF-alpha and NO production in macrophages. *Phytother Res*. 2007;21(4):347-53.
11. Prieto JM, Recio MC, Giner RM, Manes S, Giner Larza EM, Rios JL. Influence of traditional Chinese anti-inflammatory medicinal plants on leukocyte and platelet functions. *J Pharm Pharmacol*. 2003;55(9):1275-82.
12. Kim DY, Kim KH, Kim KS, Song CH, Ahn CB. The effects of Jung-wan (CV12) and Wi-su (B21) aqua-acupuncture with *Atractylodis Rhizoma* extract in gastritis. *The journal of Korean acupuncture & moxibustion society*. 1993;10(1):289-96.
13. Tian L, Huang YX, Tian M, Gao W, Chang Q. Downregulation of electroacupuncture at ST36 on TNF-alpha in rats with ulcerative colitis. *World J Gastroenterol*. 2003;9(5):1028-33.
14. Chang SL, Lin JG, Chi TC, Liu IM, Cheng JT. An insulin-dependent hypoglycaemia induced by electroacupuncture at the Zhongwan (CV12) acupoint in diabetic rats. *Diabetologia*. 1999;42(2):250-5.
15. Kitagawa H, Takeda F, Kohei H. A simple method for estimation of gastric mucus and effects of antiulcerogenic agents on the decrease in mucus during water-immersion stress in rats. *Arzneimittelforschung*. 1986;36(8):1240-4.
16. Seo MJ, Kim SJ, Kang TH, Rim HK, Jeong HJ, Um JY, et al. The regulatory mechanism of beta-eudesmol is through the suppression of caspase-1 activation in mast-cell-mediated inflammatory response. *Immunopharmacol Immunotoxicol*. 2011;33(1):178-185.
17. Kamada T, Hata J, Kusunoki H, Sugiu K, Ito M, Tanaka S, et al. Effect of long-term half-dose famotidine therapy on corpus gastritis in peptic ulcer disease. *Aliment Pharmacol Ther*. 2005;21 Suppl s2:99-104.
18. Ohta Y, Kamiya Y, Imai Y, Arisawa T, Nakano H. Plautol prevents the progression of acute gastric mucosal lesions induced by compound 48/80, a mast cell degranulator, in rats. *Pharmacology*. 2005;74(4):182-92.
19. Ohta Y, Kobayashi T, Inui K, Yoshino J, Nakazawa S. Protective effect of teprenone against acute gastric mucosal lesions induced by compound 48/80, a mast cell degranulator, in rats. *J Pharmacol Sci*. 2003;93(3):337-46.
20. Joo JH. Dangaesimbub. Seoul: Dae Sung Publishing Company, 1982:503-9.
21. Moriga M, Aono M, Murakami M, Uchino H. The role of gastric mucosal hexosamine in aspirin-induced ulcers. *Gastroenterol Jpn*. 1980;15(1):1-6.
22. Katsurahara M, Kobayashi Y, Iwasa M, Ma N, Inoue H, Fujita N, et al. Reactive nitrogen species mediate DNA damage in *Helicobacter pylori*-infected gastric mucosa. *Helicobacter*. 2009;14(6):552-8.
23. Yazici I, Turkan I, Sekmen AH, Demiral T. Salinity tolerance of purslane (*Portulaca oleracea* L.) is achieved by enhanced antioxidative system, lower level of lipid peroxidation and proline accumulation. *Environ Exp Bot*. 2007;61(1):49-57.
24. Kwon K, Park HS. The effect of lumbricus pharmacopuncture on the lipid lowering, anti-oxidative activity and concentration

- ion of proinflammatory cytokines in rats fed high fat diet. *The journal of Korean acupuncture & moxibustion society*. 2010;27(2):59-69.
25. Jung YO. An update on the management of hyperuricemia and gout. *Korean J Med*. 2009;76(5):538-43.
 26. Shin JS, Moon JY. Effect of chilgitang extract for herb-acupuncture on ROS, RNS, Lipid Peroxidation and LDL oxidation. *Korean Journal of Acupuncture*. 2008;25(4):89-104.