The Journal of Veterinary Medical Science

Preventive effects of Chinese Herb Chai-Hu-Gui-Zhi-Tang Extract on Water Immersion Restraint Stress-Induced Acute Gastric Ulceration in Rats

Journal:	*The Journal of Veterinary Medical Science*
Manuscript ID:	JVMS-09-0284.R3
Manuscript Type:	Full Paper
Date Submitted by the Author:	
Complete List of Authors:	Chen, Chi-Yang; National Taiwan University, Graduate Institute of Veterinary Medicine Kuo, Tsung-Li; National Taiwan University, Department of Forensic Medicine Sheu, Shi-Yuan Kuo, Tzong-Fu; National Taiwan University, Graduate Institute of Veterinary Medicine
Keywords:	Chai-hu-gui-zhi-tang, Gastric ulcer, Water immersion restraint stress, Non-protein sulfhydryls, Antioxidant effect
Category:	Pharmacology

Title：Preventive effects of Chinese Herb Chai-Hu-Gui-Zhi-Tang Extract on Water Immersion Restraint Stress-Induced Acute Gastric Ulceration in Rats

Categories：Pharmacology; Biochemistry

Type：Full paper

Running head：Chai-Hu-Gui-Zhi-Tang for gastric ulceration therapy

Authors：

Chi-Yang Chen	Graduate Institute of Veterinary Medicine, College of Bio-Resources and Agriculture, National Taiwan University No.1, Sec. 4, Roosevelt Rd., Taipei, Taiwan 106, R.O.C.
Tsung-Li Kuo	Department of Forensic Medicine, College of medicine, National Taiwan University No.7, Jhongshan S. Rd., Taipei, Taiwan 100, R.O.C.
Shi-Yuan Sheu	[1]School of Chinese Medicine, China Medical University No.91, Hsueh-Shih Road, Taichung, Taiwan 404, R.O.C. [2]Department of Acupuncture, E-DA Hospital/I-Shou University No.1 E-Da Road, Jiau-shu Tsuen, Yan-Chau Shiang, Kaohsiung County, Taiwan 824, R.O.C.
Tzong-Fu Kuo* Corresponding author	Graduate Institute of Veterinary Medicine, College of Bio-Resources and Agriculture, National Taiwan University No.1, Sec. 4, Roosevelt Rd., Taipei, Taiwan 106, R.O.C. Fax : 886-2-23661451 E-Mail：tzongfu@ntu.edu.tw

Preventive effects of Chinese Herb Chai-Hu-Gui-Zhi-Tang Extract on Water Immersion Restraint Stress-Induced Acute Gastric Ulceration in Rats

Chi-Yang Chen [1], Tsung-Li Kuo [2], Shi-Yuan Sheu [3,4] and Tzong-Fu Kuo [1,*]

[1] Graduate Institute of Veterinary Medicine, College of Bio-Resources and Agriculture, National Taiwan University, Taipei, Taiwan, 106, ROC

[2] Department of Forensic Medicine, College of medicine, National Taiwan University, Taipei, Taiwan, 106, ROC

[3] School of Chinese Medicine, China Medical University, Taichung City, Taiwan, 404, ROC

[4] Department of Acupuncture, E-DA Hospital / I-Shou University, Kaohsiung County, Taiwan, 824, ROC

* Corresponding author：Professor Tzong-Fu Kuo

Department and Graduate Institute of Veterinary Medicine, National Taiwan University

No.1, Sec. 4, Roosevelt Rd., Taipei, Taiwan 106, Republic of China

http://www.vm.ntu.edu.tw/

TEL: 886-2-33661295 TEL with FAX: 886-2-23661451

E-Mail: tzongfu@ntu.edu.tw

ABSTRACT

It is well known that maintenance therapy of Chai-hu-gui-zhi-tang (CHGZT), a traditional Chinese medicine, has been proven to prevent the recurrence of peptic ulcers. However, little is known as to whether or not the prescription shows protective effects against acute gastric injury. In the present study, we investigated the preventive effects of pretreatment with CHGZT extract on the development of water immersion restraint stress-induced acute gastric ulceration in male Wistar rats. The CHGZT extract (50, 250, 500 mg/kg b.w., *p.o.*) was given to the rats before they were exposed to 2 or 4 h of water immersion restraint stress and then were sacrificed immediately after the stress exposure. The gastric mucosal lesions were evaluated macroscopically and the gastric mucosal, and hepatic non-protein sulfhydryls (NP-SH) were measured simultaneously. The results indicate that exposure to water immersion restraint stress resulted in the development of acute gastric stress erosions. Pretreatment with CHGZT extract caused a significant reduction of stress lesions and an increase in gastric mucosal NP-SH and hepatic NP-SH concentrations. We conclude that the anti-ulcer response and the extensive antioxidant effect of Chai-hu-gui-zhi-tang may be valuable to the prevention of experimental gastric mucosal lesions in rats because it possesses preventive and gastroprotective effects.

Keywords: Chai-hu-gui-zhi-tang (CHGZT); Gastric ulcer; Water immersion restraint stress; Non-protein sulfhydryls; Antioxidant effect

INTRODUCTION

Chai-hu-gui-zhi-tang (CHGZT), a traditional Chinese herbal prescription, is a mixture of nine herbs; *Bupleuri radix*, *Scutellariae radix*, *Ginseng radix*, *Cinnamomi ramulus*, *Paeoniae radix*, *Zingiberis rhizome*, *Pinelliae tuber*, *Glycyrrhizae radix* and *Zizyphi fructus*has, has shown a good antioxidant effect and has been used to prevent the formation of nervous lesions, such as epilepsy, Alzheimer's disease and developmental defects of neurons during pregnancy and after birth. [12, 25]. In traditional Chinese medicine, CHGZT has been used for the therapy of gastric ulcers and gastritis, but the underlying mechanisms are not well understood. Among the herbal drugs composed in GHGZT, *Scutellariae radix* [6] and *Panax ginseng* [11] have been used extensively and the clinical efficacies were documented with anti-ulcer activity. The flavonoids of *Scutellariae radix* have been found to be free radical scavengers [3]; free radicals play an important role in ulcerative and erosive lesions of the gastrointestinal tract. Glycyrrhizae radix water extract and its two major constituents, glycyrrhizin and 3-glycyrrhetinic monodesmoside, may be promising for amelioration of hypoxia (ischemia)-reoxygenation (reperfusion) injury and improvement of renal function by acting as antioxidant and oxygen radical-scavenging agents [32].

It is well known that stress plays a major role as a risk factor in the occurrence of stomach erosion and ulcers [22]. Water immersion restraint stress (WIRS) mimics the clinical acute gastric ulcerations caused by trauma, surgery, or sepsis [8] and has been widely accepted for studying stress ulcers [5, 26, 27]. Involvement of central nervous stem compounds such as the limbic system, hypothalamus and brain stem nuclei have been considered [9]. Mucosal ischemia and enhanced back diffusion of hydrogen ions

have been proposed as major local mechanisms for these stress-induced injuries [29]. Gastric acid secretion is remarkably enhanced under water immersion stress without significant alteration in gastric mucosal blood flow [10]. Ohta *et al*. have shown the therapeutic effect of Oren-gedoku-to extract on stress-induced acute gastric mucosal lesions in rats, which indicated the preventive actions on lipid peroxidation and sulphydryl oxidation via oxygen free radicals generated by the xanthine-XO system and infiltrated neutrophils in the gastric mucosa and on neutrophil infiltration into the tissue [17]. Yoshikawa *et al*., indicated that Saiko-keishi-to (Chai-hu-gui-zhi-tang) extract not only had an ability to scavenge oxygen free radicals, but also a protective effect against ischaemia-reperfusion-induced gastric mucosal lesions, mediated by oxygen free radicals and lipid peroxidation in rats [34].

The present study was performed to investigate the preventive effects of CHGZT against acute gastric mucosal lesions development of water immersion restraint stress in rats. The effect of pretreatment of CHGZT extract on the progression of gastric mucosal lesions in rats subjected to stress exposures of 2 and 4 h periods, and the gastric mucosal lesions were evaluated macroscopically (ulcer index). The gastric mucosal and hepatic non-protein sulfhydryls (NP-SH), which are a major source of cellular reducing substances, were measured for the purpose of evaluating the preventive and gastroprotective effects of gastric mucosal injury.

MATERIALS AND METHODS

Materials.

Chai-hu-gui-zhi-tang (CHGZT) was provided by Kaiser Pharmaceutical Co. Ltd. (Taipei, Taiwan) and was prepared from boiled water extracts of the following herbs: *Bupleuri radix* of 1.5 g, *Scutellariae radix* of 0.6 g, *Ginseng radix* of 0.6 g, *Cinnamomi ramulus* of 0.6 g, *Paeoniae radix* of 0.6 g, *Zingiberis rhizome* of 0.6 g, *Pinelliae tuber* of 0.9 g, *Glycyrrhizae radix* of 0.4 g and *Zizyphi fructus* of 0.4 g. The total weight was 6.0 g. All chemicals were ACS reagent grade. Butylated hydroxytoluene (BHT), 5,5'-dithiobis (2-nitrobenzoic acid) (DTNB), ethylenediamine-tetraacetic acid disodium (EDTA-Na_2) and trichloroacetic acid were commercially available from Sigma (St. Louis, MO, USA). Methanol and other solvents were analytical and supplied by J.T. Baker (Phillipsburg, NJ, USA). Deionized water was obtained from a Milli-Q Plus analytical deionization system (Bedford, MA, USA).

Induction of Gastric Ulceration.

Male Wistar rats weighting 260-280 g were purchased from the laboratory animal center, College of Medicine, National Taiwan University (Taipei, Taiwan). All experimental procedures were approved by the Institutional Animal Ethics Committee of National Taiwan University, and conducted in accordance with the internationally accepted principles for laboratory animal used and care. Rats were starved for 18 h but were allowed free access to water, before the induction of WIRS or the sampling of their gastric mucosa. Each rat was subjected to WIRS as described previously by Takagi and Okabe [26]. The rats were restrained in firmly fitted restraint cages ($6 \times 7 \times 20$ cm^3) and

115 vertically immersed in water maintained at 23℃ to the level of the xiphoid process for 2
116 or 4 h to induce gastric mucosal lesions (Fig 1).
117
118 (Insert Fig 1)
119
120 Treatment with Chinese Herb Chai-Hu-Gui-Zhi-Tang Extract.
121 To investigate the preventive effects of CHGZT on WIRS induced gastric mucosal
122 lesions, rats were pretreated with different doses of CHGZT extract suspension (50, 250
123 and 500 mg/kg b.w., *p.o.*) before a 2 or 4 h induction of WIRS. All control groups of this
124 study were treated with deionized water, 0.5 mL/100 g b.w., *p.o*. Each rat was sacrificed
125 under CO_2 anesthesia after treatment and the stomaches were quickly removed. Gastric
126 mucosal lesions were examined under a dissecting microscope (×10), and the values of
127 the ulcer index (UI) was expressed by measuring the total length (cm) of all gastric
128 mucosal lesions in the stomaches induced during the stress [26]. Histologic assessments
129 were made with photomicroscope. Specimens from normal and abnormal gastric tissues
130 were fixed in 10% formaldehyde, routinely dehydrated, cleaned, infiltrated with wax,
131 embedded and made into serial 4-μm thick sections. The sections were dewaxed, stained
132 with haematoxylin and eosin technique.
133
134 Biochemical Assays.
135 Samples used for assays of gastric mucosa and hepatic non-protein sulfhydryls
136 (NP-SH) were prepared with Ellman's reagent [19] using 5,5'-dithiobis (2-nitrobenzoic
137 acid) as described previously by Sedlak and Lindsay. The gastric mucosae were collected

with a glass slide on ice bath and the liver was quickly removed from the rat after sacrifice. 200 mg of gastric mucosa or liver tissue was immediately homogenized with 2 mL 0.02 M EDTA-Na_2 containing 0.002 % BHT in a Teflon-glass homogenizer on ice bath.

Statistical Evaluation.

All results were presented as the mean ± SD. The paired data were compared by Student's *t*-test and the significant level was set at $P<0.05$.

RESULTS

Assessment of Stress-Induced Gastric Mucosal Damage

As the period of stress was increased from 2 to 4 h, the values of the ulcer index and severity of the ulcers were increased as shown in Fig 2. No macroscopic lesions were observed in untreated rats. After the stress extensive superficial erosions with various lengths and congestion of the surrounding portion were observed in the gland area of the stomach. Appearance of gastric ulcers in the glandular area when examined, and the blood coagulums can be seen at the base of ulcers.

(Insert Fig 2)

At the histological examination, all of the lesions were erosion of mucosa and they extended down to submucosae with neutrophils infiltration. Obvious blood stasis performance appeared in stressed rats (Fig 3).

(Insert Fig 3)

Changes in Ulcer Index in Stress-Induced Gastric Mucosal Damage by CHGZT Pretreatment

When rats were subjected to WIRS for 2 and 4 h, the values of the ulcer index increased dramatically after stress as compared with the untreated rats (Fig 4). The value of the ulcer index at a stress of 4 h was 1.43 ± 0.21 cm and this value was 2.2-fold larger than that at a stress of 2 h. When rats were pre-treated with CHGZT of 50, 250 and 500 mg/kg b.w., *p.o.*, before the onset of WIRS, the administration of each dose of the medicine significantly prevented the progression of gastric mucosal lesions. The values of the ulcer index decreased markedly in the stress-loaded rats ($P < 0.01$).

(Insert Fig 4)

Changes in Nonpotein Sulfhdryls in Stress-Induced Gastric Mucosal Damage by CHGZT Pretreatment

Effects of CHGZT pretreatment followed by the onset of WIRS (2 and 4 h), the changes in gastric mucosal and hepatic NP-SH concentrations in rats, shown in Fig 5, were obtained. Gastric mucosal NP-SH concentration in rats with 2 and 4 h of WIRS were significantly lower than that in control rats without WIRS. The decreased concentration of gastric mucosal NP-SH at WIRS after 2 and 4 h were both recovered by the CHGZT pretreatment. When administered at the dose of 50, 250, and 500 mg/kg b.w.,

p.o, the average recovered gastric mucosal NP-SH concentrations were 1.2-, 1.5-, and 1.5-fold, respectively, higher than the concentration at a stress of 2 h. Similarly, data showed that the recovered gastric mucosal NP-SH concentrations were 1.2-, 1.8-, and 1.5-fold, respectively, higher than that concentration with 4 h of the stress. As hepatic NP-SH concentrations of rats subjected to WIRS for 2 and 4 h were slightly decreased of those of control rats without the stress, the decreased concentration of hepatic NP-SH at WIRS were also recovered by the CHGZT pretreatment. This pre-CHGZT administration prevented not only gastric mucosal lesion development in rats subjected to the stress, but also increased gastric mucosal and hepatic NP-SH concentrations in the rats subjected to 2 and 4 h of the stress.

(Insert Fig 5)

DISCUSSION

The Chinese formula Chai-hu-gui-zhi-tang (CHGZT) has been used to treat influenza, pleurisy, stomach ache, gastroxia (hyperacidity), stomach ulcer, duodenum ulcer, epilepsy [12, 25], hepatitis [21], pancreatitis [24], jaundice, pain between the ribs, and pain of sciatica, etc. The results from the animal models indicated that CHGZT extract has antioxidant effects and has a scavenging activity for free radicals generated within an iron-induced epileptogenic regions of a rat's brain [12]. CHGZT can inhibit pepsin and stomach acid secretion, can enhance the secretion of stomach mucous, can promote blood circulation in the stomach and can improve the protection of the stomach [7]. Recently, Yasukawa et al. reported that reactive oxygen species (ROS) are associated

with gastric ulcer [31]. Uteshev *et al*. indicated that stronger intensity of lipid peroxidation and less activity of the antioxidant system of blood are correlated with gastroduodenal ulcerous hemorrhages [28]. As a result of experimental gastric ulcers showed, the elimination of free radicals by anti-ulcer agent that has antioxidant and free-radical scavenging activities, may contribute to the reduction of severity in ulcer recurrence [15, 18].

The present study provided evidence that orally administered CHGZT extract at a dose of 50, 250, 500 mg/kg b.w., *p.o.* can prevent the progression of acute gastric mucosal lesions in rats subjected to WIRS of a 2 and 4 h period. According to a report by Nishida *et al*., the progression of WIRS induced acute gastric mucosal lesions in rats was mainly related to enhance gastric mucosal sulfhydryl oxidation and lipid peroxidation [16]. Decreased gastric mucosal non-protein sulfhydryls concentration found at the second and the fourth hour after WIRS, significantly recovered by the oral administration of CHGZT extract (250 and 500 mg/kg b.w., *p.o.*), and the recovered concentration was higher than the concentration found at 2 and 4 h of the stress. It has been implicated that sulfhydryls in a stomach is important for the maintenance of gastric mucosal integrity [14].

Many studies have shown that oxygen free radicals are implicated as mediators of gastric mucosal injury. The mucosal availability of the antioxidant reduced glutathione (GSH, γ-glutamyl-cysteinyl glycine)) which is an important protective factor against the development of gastric mucosal ischemia/reperfusion injury [23]. Body *et al*. reported on gastric glutathione depletion and acute ulcerogenesis by diehtylmaleate given subcutaneously to rats [4]. For the oxidation of reduced glutathione and the formation of

mixed disulfides between protein and non-protein sulfhydryls, it was well known that non-protein sulfhydryls (NP-SH) was a major source of cellular reducing substances. Recently, Nagy *et al*. indicated that endogenous sulfhydryls (SH) plays an important role in the maintenance of gastroduodenal integrity and in the protection against chemically-induced lesions in cells, tissues and organs [14]. Furthermore, Ohta *et al*. suggested that the therapeutic effect of Oren-gedoku-to extract (Huanglian-Jiedu-Tang), a traditional Chinese herbal medicine, could be due to the preventive actions on lipid peroxidation and sulfydryl oxidation via oxygen free radicals generated by the xanthine-XO system [17]. Antioxidant defense mechanism may therefore be of critical importance in protecting against the development of acute gastric mucosal injury.

It was widely accepted that a major portion of phospholipid peroxidation occurs due to the generation of oxygen-derived free radicals. Previous investigations demonstrated the prevention of oxidative damage of the gastric mucosa by significantly blocking lipid peroxidation and by scavenging the endogenous hydroxyl radical ($^{\cdot}$OH), which is the major causal factor for the formation of an ulcer [2]. However, it was known that $^{\cdot}$OH-mediated oxidative caused damage to human gastric mucosal DNA. Yoshikawa *et al*. have previously reported that the gastric mucosal blood flow decreases even in the early phases of WIRS [33]. Shian *et al*. pointed out the probable role of lipid peroxidation in the pathogenesis of gastric injury induced by WIRS [20].

As described above, the herbal remedies contained in GHGZT were shown to have anti-ulcer and anti-inflammatory responses. Additionally, the constituents of *Paeoniae radix* exhibited a significant oxygen radical scavenging activity and had an inhibitory

effect on lipid peroxidation [13]. Ginger was known to stimulate digestion beneficially and has anti-ulcer effects [30]. The cytoprotective and anti-ulcerogenic effect of the ginger were shown to prevent the occurrence of gastric ulcers induced by non-steroidal anti-inflammatory drugs and hypothermic restraint stress [1].

The results in the present study indicated that orally administered CHGZT exerts a preventive effect on water immersion restraint stress-induced acute gastric ulceration in rats. Pretreatment with CHGZT markedly reduced gastric mucosal lesions in the stress-loaded rats, and the decreased gastric mucosal and hepatic NP-SH concentrations after the onset of WIRS were recovered. The endogenous sulfhydryls play an important role in the maintenance of gastroduodenal integrity; the relatively high concentration of non-protein sulfhydryls in the gastric mucosa also indicates their possible implications for gastroprotection. Since an antioxidant defense mechanism may be critical important in protecting the development of acute gastric mucosal injury, the anti-ulcer response and the extensive antioxidant effect of CHGZT may be valuable to the prevention, which possesses preventive and gastroprotective effects on experimental gastric mucosal lesions in rats.

Acknowledgements

This work was financially supported by the National Council of Science, Executive Yuan, Taiwan, R.O.C (NSC91-2313-B-002-382).

REFERENCES

1. Al-Yahya, M. A., Rafatullah, S., Mossa, J. S., Ageel, A. M., Parmar, N. S. and Tariq, M. 1989. Gastroprotective activity of ginger zingiber officinale rosc., in albino rats. *Am. J. Chin. Med.* 17: 51–56.
2. Bandyopadhyay, U., Biswas, K., Chatterjee, R., Bandyopadhyay, D., Chattopadhyay, I., Ganguly, C. K., Chakraborty, T., Bhattacharya, K. and Banerjee, R. K. 2002. Gastroprotective effect of Neem (Azadirachta indica) bark extract: Possible involvement of H^+-K^+-ATPase inhibition and scavenging of hydroxyl radical. *Life Sci.* 71: 2845–2865.
3. Bochorakova, H., Paulova, H., Slanina, J., Musil, P. and Taborska, E. 2003. Main flavonoids in the root of Scutellaria baicalensis cultivated in Europe and their comparative antiradical properties. *Phytother. Res.* 17: 640–644.
4. Body, S. C., Sasame, H. A. and Body, M. R. 1981. Gastric glutathione depletion and acute ulcerogensis by diehtylmaleate given subcutaneously to rats. *Life Sci*. 28: 2987–2992.
5. Brzozowski, T., Konturek, S. J., Sliwowski, Z., Pytko-Polonczyk, J., Szlachcic, A. and Drozdowicz, D. 1996. Role of capsaicin-sensitive sensory nerves in gastroprotection against acid-independent and acid-dependent ulcerogenesis. *Digestion* 57: 424–432.
6. Chi, Y. S., Lim, H., Park, H. and Kim, H. P. 2003. Effects of wogonin, a plant flavone from Scutellaria radix, on skin inflammation: in vivo regulation of inflammation-associated gene expression. *Biochem. Pharmacol*. 66: 1271–1278.

7. Ding, J., Chen, J. Z. and Li, Z. Y. 2007. An experimental study on the structural and functional changes of gastric mucosa in rats with liver-qi stagnation syndrome. *Liaoning J. Tradit. Chin. Med*. 34: 229–232.

8. Ernst, H., Konturek, P. C., Brzozowski, T., Lochs, H., Hahn, E. G. and Konturek, S. J. 1998. Adaptation of gastric mucosa to stress. Effect of ranitidine. *J. Physiol. Pharmacol*. 49: 405–419.

9. Glavin, G. B., Murison, R., Overmier, J. B., Pare, W.P., Bakke, H. K., Henke, P.G. and Hernandez, D. E. 1991. The neurobiology of stress ulcers. *Brain Res. Brain Res. Rev*. 16: 301–343.

10. Hayase, M. and Takeuchi, K. 1986. Gastric acid secretion and lesion. Formation in rats under water-immersion stress. *Dig. Dis. Sci*. 31: 166–171.

11. Jeong, C. S., Hyun, J. E. and Kim, Y. S. 2003. Ginsenoside Rb1: the anti-ulcer constituent from the head of Panax ginseng. *Arch. Pharm. Res*. 26: 906–911.

12. Komatsu, M., Hiramatsu, M., Yokoyama, H. and Willmore, L. J. 1996. Effect of TJ-960 (a Japanese herbal medicine) on free radical changes within an iron-induced focal epileptogenic region in rat brain measured by in vivo L-band electron spin resonance. *Neurosci*. *Lett*. 205: 189–192.

13. Lee, S. C., Kwon, Y. S., Son, K. H., Kim, H. P. and Heo, M. Y. 2005. Antioxidative constituents from Paeonia lactiflora. *Arch. Pharm. Res*. 28: 775–783.

14. Nagy, L., Nagata, M. and Szabo, S. 2007. Protein and non-protein sulfhydryls and disulfides in gastric mucosa and liver after gastrotoxic chemicals and sucralfate: Possible new targets of pharmacologic agents. *World J. Gastroenterol*. 13: 2053–2060.

15. Naito, Y., Yoshikawa, T., Iinuma, S., Yagi, N., Matsuyama, K., Boku, Y., Fujii, T., Yoshida, N., Kondo, M. and Sasaki, E. 1998. Rebamipide protects against indomethacin-induced gastric mucosal injury in healthy volunteers in a double-blind, placebo-controlled study. *Dig. Dis. Sci*. 43: 83S–89S.

16. Nishida, K., Ohta, Y., Kobayashi, T. and Ishiguro, I. 1997. Involvement of xanthine oxidase system and neutrophils in the development of acute gastric mucosal lesions in rats with water immersion restraint stress. *Digestion* 58: 340–351.

17. Ohta, Y., Kobayashi, T., Nishida, K., Nagata, M. and Ishiguro, I. 1999. Therapeutic effect of Oren-gedoku-to extract on stress-induced acute gastric mucosal lesions in rats. *Phytother. Res*. 13: 588–592.

18. Podoprigorova, V. G., Khibin, L. S., Kozlov, N. B. and Barsel, V. A. 1999. Effectiveness of synthetic antioxidants in treatment of patients with peptic ulcer an open randomized controlled trial. *Klin. Med*. 77: 32–35.

19. Sedlak, J. and Lindsay, R. H. 1968. Estimation of total, protein-bound, and nonprotein sulfhydryl groups in tissue with Ellman's reagent. *Anal. Biochem*. 25: 192–205.

20. Shian, W. M., Sasaki, I., Kamiyama, Y., Naito, H., Matsuno, S. and Miyazawa, T. 2000. The role of lipid peroxidation on gastric mucosal lesions induced by water-immersion-restraint stress in rats. *Surg. Today* 30: 49–53.

21. Shimizu, I. 2000. Sho-saiko-to: Japanese herbal medicine for protection against hepatic fibrosis and carcinoma. *Eur. J. Gastroenterol. Hepatol*. 15: D84–D90.

22. Silen, W., Merhav, A. and Simson, J. 1981. The pathophysiology of stress ulcer disease. *World J. Surg.* 5: 165–174.

23. Stein, H. J., Hinder, R. A. and Oosthuizen, N. M. J. 1990. Gastric mucosal injury caused by hemorrhagic shock and reperfusion: Protection role of the antioxidant glutathione. *Surgery* 108: 467–474.

24. Su, S. B., Motoo, Y., Iovanna, J. L., Xie, M. J., Taga, H. and Sawabu, N. 2001. Antifibrotic effect of the herbal medicine Saiko-keishi-to (TJ-10) on chronic pancreatitis in the WBN/Kob rat. *Pancreas* 22: 8–17.

25. Sugaya, E., Sugaya, A., Kajiwara, K., Yuyama, N., Tsuda, T., Motoki, M., Shimizu Nishikawa, K. and Kimura, M. 1997. Nervous diseases and Kampo (Japanese herbal) medicine: a new paradigm of therapy against intractable nervous diseases. *Brain Dev*. 19: 93–103.

26. Takagi, K. and Okabe, S. 1968. The effects of drugs on the stress ulcer in rats. *Jpn. J. Pharmacol*. 18: 9–18.

27. Uramoto, H., Ohno, T. and Ishihara, T. 1990. Gastric mucosal protection induced by restraint and water-immersion stress in rats. *Jpn. J. Pharmacol*. 54: 287–298.

28. Uteshev, N. S., Pakhomova, G. V., Tveritneva, L. F., Lebedev, A. G., Matveev, S. B., Marchenko, V. V. and Golikov, P. P. 1999. Lipid peroxidation and antioxidant system functions in patients with gastroduodenal ulcerous hemorrhages undergoing conservative and surgical treatment. *Vestn. Khir. Im. I. I. Grek*. 158: 24–26.

29. Yabana, T. and Yachi, A. 1988. Stress-induced vascular damage and ulcer. *Dig. Dis. Sci*. 33: 751–761.

30. Yamahara, J., Mochizuki, M., Rong, H. Q., Matsuda, H. and Fujimura, H. 1988. The anti-ulcer effect in rats of ginger constituents. *J. Ethnopharmacol*. 23: 299–304.

31. Yasukawa, K., Kasazaki, K., Hyodo, F. and Utsumi, H. 2004. Non-invasive analysis of reactive oxygen species generated in rats with water immersion sestraint-induced gastric lesions using in vivo electron spin resonance spectroscopy. *Free Radic. Res.* 38: 147–155.

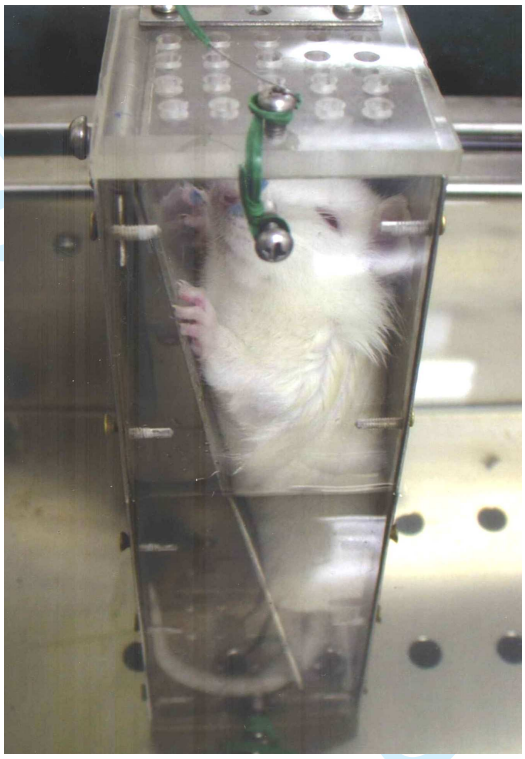
32. Yokozawa, T., Liu, Z. W. and Chen, C. P. 2000. Protective effects of Glycyrrhizae radix extract and its compounds in a renal hypoxia (ischemia)-reoxygenation (reperfusion) model. *Phytomedicine*. 6: 439–445.

33. Yoshikawa, T., Miyazawa, H., Yoshida, N., Sugino, S. and Kondo, M. 1986. Increase in lipid peroxidation in rat gastric musical lesions induced by water-immersion restraint stress. *J. Clin. Biochem. Nutr*. 1: 271–277.

34. Yoshikawa, T., Takahashi, S. and Kondo, M. 1991. Oxygen derived free radical and traditional Kampo medicines. *Rec. Adv. Cardiovasc. Dis*. 12: 173–181.

Fig 1.

Each rat was immobilized the stress cage (6 × 7 × 20 cm^3) which was immersed vertically in a water bath kept on 23℃ for 2 or 4 h to the height of the xiphoid of the Wistar rat to produce deeper ulceration.



379 Fig 2.

380 Appearance of gastric mucosal lesions induced by water-immersion restraint stress. The

381 gastric mucosa of a control rat (a) and the blood coagulums were marked at the base of

382 ulcers by treatment with water-immersion stress (2 hr: b; 4 hr: c)

(a) (b) (c)

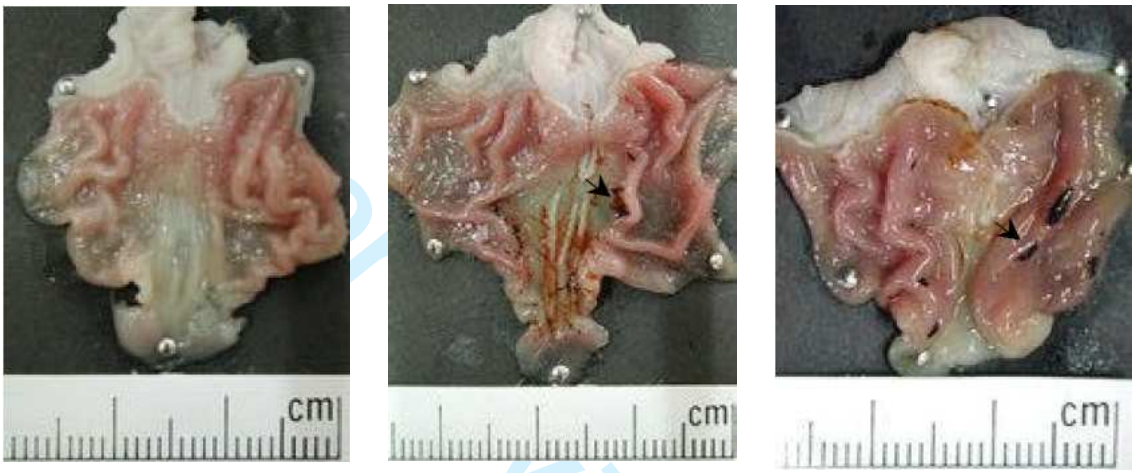


Fig 3.

Microphotograph showing the gastric mucosa from a control rat (a) and a rat subjected to water immersion-restraint stress for 4 h (b). Note that the blood stasis and neutrophil infiltration were observed in the pathogenesis of gastric mucosal lesions induced by water immersion restraint stress. (H&E staining. Scale bar = 50 μm. A: Blood stasis; B: Neutrophil infiltration)

(a) (b)

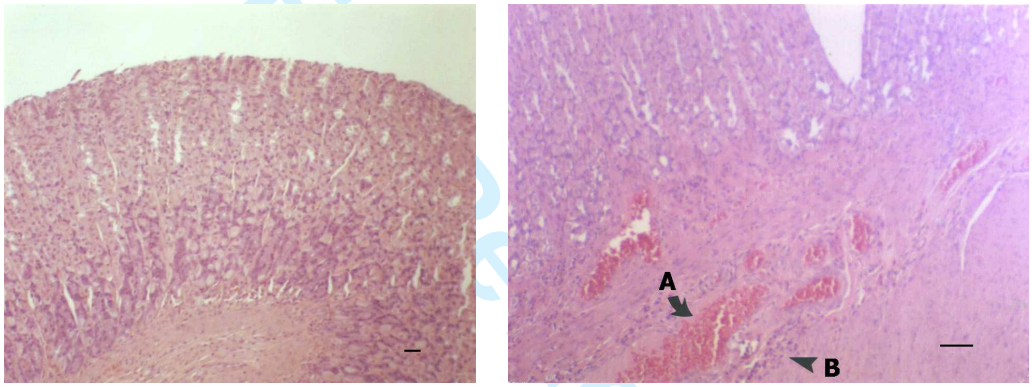


Fig 4.

Effect of pretreatment with *Chai-hu-gui-zhi-tang* extract on the development of gastric mucosal lesions in rats with water immersion restraint stress over a 2-h or 4-h period. Rats received oral administration of CHGZT (50, 250, 500 mg/kg) prior to water immersion restraint induction. Rats without CHGZT pretreatment received oral administration of equal volume of deionized water. These rats were subjected to water immersion restraint stress for 2 or 4 h. Each value represents the mean ± SD for 10 animals. Significant differences were calculated from Student's *t*-test. ※$P < 0.05$, ※※$P < 0.01$ *versus* the controls; $^{*}P < 0.05$, $^{**}P < 0.01$ versus the control stressed rats with WIRS treatment.

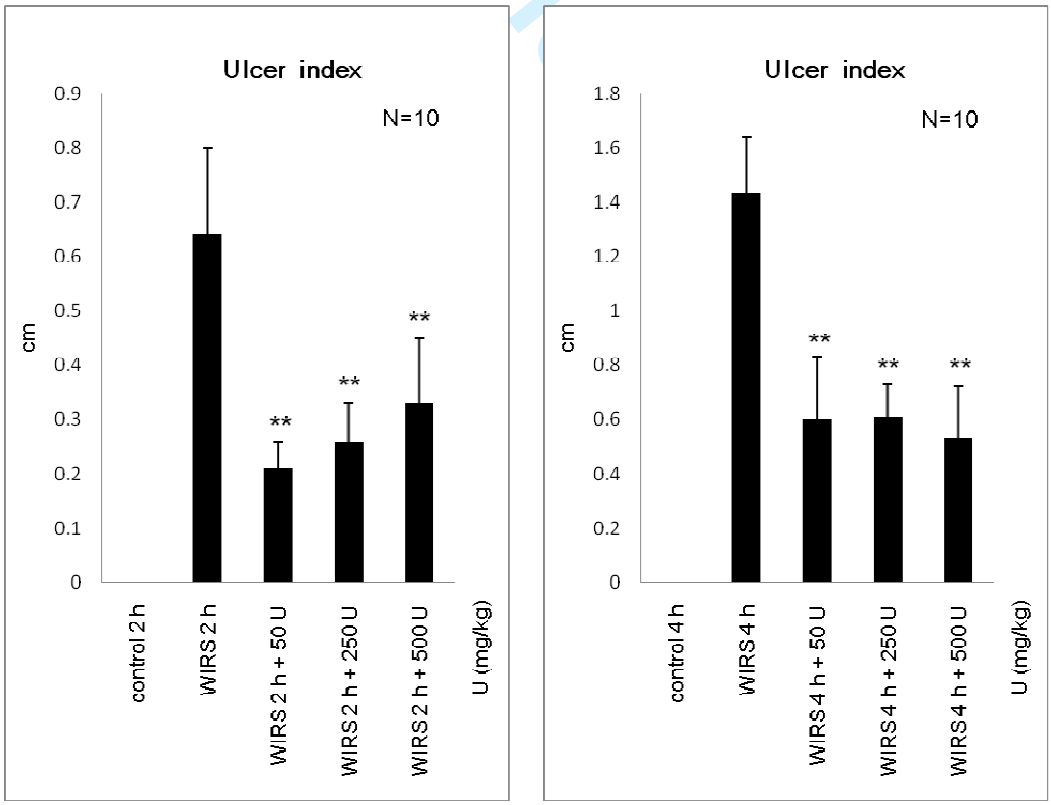
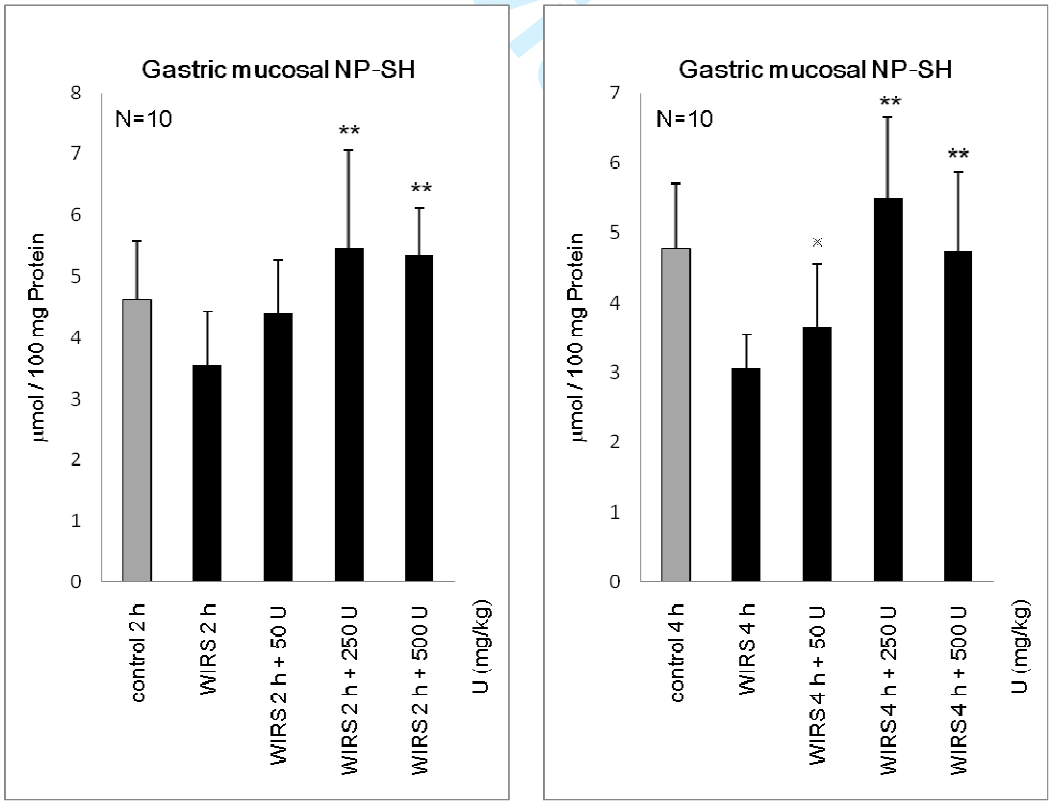


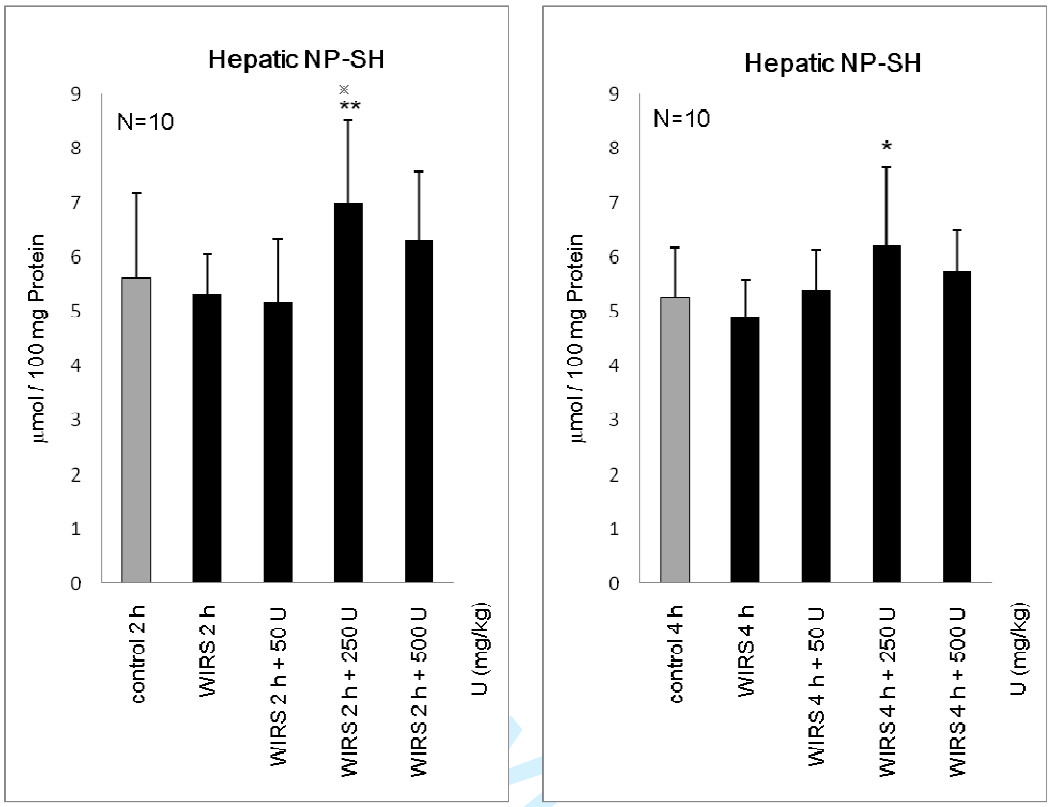
Fig 5.

Effect of pretreatment with *Chai-hu-gui-zhi-tang* extract on the changes in gastric mucosal NP-SH (a) and hepatic NP-SH (b) concentrations in rats with water immersion restraint stress over a 2-h or 4-h period. Rats received oral administration of CHGZT (50, 250, 500 mg/kg) prior to water immersion restraint induction. Rats without CHGZT pretreatment received oral administration of equal volume of deionized water. These rats were subjected to water immersion restraint stress for 2 or 4 h. Each value represents the mean ± SD for 10 animals. Significant differences were calculated from Student's *t*-test. ※$P < 0.05$, ※※$P < 0.01$ *versus* the controls; $^{*}P < 0.05$, $^{**}P < 0.01$ versus the control stressed rats with WIRS treatment.

(a)



416 (b)



417