

Full Length Research Paper

Studied Chinese herbs on *Helicobacter pylori*

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Helicobacter pylori (*H. pylori*) are a bacterium implicated in the etiology of stomach cancer and ulcers. Epidemiology of the antibiotic resistance of *H. pylori* appears to be higher. The study was to screen an efficiency substance of Chinese herbs, which could be used in preventing and treating *H. pylori* diseases. A total of 40 extracted efficiency substances from Chinese herbs were examined and screened for anti-*H. pylori* activity performed on 96 microwell plate. 45 cases of chronic gastritis with *H. pylori* infected were treated by a prescription based on Chinese herbs of "clear heat and eliminate toxins". It is showed that all except 15 extract from 40 plants were identified as having potent anti-*H. pylori* activities. But the most active extracts were those from Gallnut (cocoon) of Chinese Sumac (MIC: <1:512), Clover Flower Bud (MIC: 1:256), Houtuynia (MIC: 1:512), Agrimony (MIC: 1:256) Coptis Rhizome (MIC: <1:512). Subsequently, a detailed comparative study of the extract of the active herbs was performed to anti-*H. pylori*. Amongst the active plants the inhibitory properties of *H. pylori* were found prominent. Gallnut (cocoon) of Chinese Sumac, Clover Flower Bud, Houtuynia, Agrimony and Coptis Rhizome possess higher potent anti-*H. pylori* activity.

Key words: Chinese herbs, extract, *Helicobacter pylori*, comparative study, activity.

INTRODUCTION

The World Health Organization declared *H. pylori* a class I carcinogen because of the association of *H. pylori* and gastric malignancies (Gold, 1999). There is mounting evidence that *H. pylori* infection plays an important role in the pathogenesis of carcinoma of the gastric antrum and fundus. *H. pylori* infection is also considered the chief etiological factor of ulcer disease (Vergara et al., 2002). Most cases of chronic gastric ulcers and many cases of atrophic gastritis are due to *H. pylori* infection (Malhotra and Pandhi, 2002). Epidemiologic studies show that patients with gastric carcinoma have a higher incidence of *H. pylori* infection at the time of diagnosis compared to a control population (Hirai et al., 1999). Antibiotic therapy and a combination of two or three drugs have been widely used to eradicate these infections. Therapies include triple therapy combining metronidazole, omeprazole and clarithromycin, which often has better patient compliance than the more complicated standard triple therapy

regimen (Adamsson et al., 1999). The dual therapy combination of omeprazole and clarithromycin, has been submitted to the Food and Drug Administration (FDA). Cure rates in clinical trials have ranged from 70 to 83% (David and Kearney, 2003). Sometimes adverse effect could be found after dual therapy or triple therapy.

Development of drug resistance in bacteria calls for new sources of drugs, and plants seem to be a logical source of new antibacterial compounds. The aim of this study was to evaluate the effects of herbal medicines in treating *H. pylori* as a possible agent.

MATERIALS AND METHODS

Preparation of extracts

The plant parts purchased from Affiliated Shanghai Ninth People's Hospital were showed in Table 1, which selected ground, immersed in deionized water (10 g/50 ml), decocted for twice, merged the juice water, decompression concentrated as herb agent, and the residue directly assayed against the microorganisms that

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Table 1. Effect of Chinese herbs on *H. pylori*.

Common Name	Botanical Name	MIC	Common Name	Botanical Name	MIC
Indigo (Isatis) leaf processed	<i>Indigo Pulverata Levis (powder)</i>	>1:1	Acanthopanax Root Bark	<i>Acanthopanax Gracilistylus Cortex Radix</i>	>1:1
Gallnut (cocoon) of Chinese Sumac	<i>Rhus Chinese's Galla</i>	<1:512	Eupatorium	<i>Eupatorium Fortunei Herba</i>	>1:1
Ampelopsis root	<i>Ampelopsis Radix</i>	1:8	Gingko Nut	<i>Gingko Bilobae Semen</i>	1:16
Angelica root	<i>Angelica Dahurica Radix</i>	1:2	Torreya Seeds	<i>Torreya Grandis Semen</i>	1:16
Honeysuckle flower	<i>Lonicera Japonica Floss</i>	1:2	winter jasmine flower	<i>Jasminum Polyanthum</i>	>1:1
Field mint	<i>Mentha Haplocalyx Herba</i>	>1:1	Moutan root bark, Tree peony root bark	<i>Moutan Cortex Radix</i>	1:32
Borax	<i>Borax</i>	>1:1	Houtuynia	<i>Houttuynia Cordata Herba cum Radix</i>	1:512
Safflower flower powder	<i>Carthamus Tinctorius Flos</i>	>1:1	Ophiopogonis tuber	<i>Ophiopogonis Japonicum Tuber</i>	1:8
borneo camphor	<i>cinnamonum camphora</i>	>1:1	Patrinia	<i>Herba cum Radice Patrinia Scabiosaefolia</i>	>1:1
Shinyleaf Pricklyash root	<i>Zanthoxylum nitidum (Boxb.)</i>	>1:1	Sophora Root	<i>Sophora Flavescens Radix</i>	>1:1
pearl	<i>pearl</i>	>1:1	Amur Cork Tree Bark	<i>Phellodendri Cortex</i>	1:64
Schisandra fruit	<i>Schisandra Chinensis Fructus</i>	1:64	Hawthorn Fruit, Charred	<i>Crataegi Fructus Carbinosatum</i>	1:4
Clover flower bud	<i>Eugenia Catyophyllata Flos</i>	1:256	Sweetgum Fruit	<i>Artemisia Anomalis Herba</i>	1:2
Licorice root medium	<i>Glycyrrhizae Uralensis Radix</i>	1:16	Chrysanthemum Flower	<i>Chrysanthemum Morifolium Flos</i>	1:8
Chinese Wolfberry	<i>Lycium Barbarum Fructus</i>	1:64	Polygonum Vine	<i>Polygonum Multiflori Caulis</i>	>1:1
Cirsium root, Japanese thistle root	<i>Cirsium Japonicum Radix</i>	1:2	Agrimony	<i>Agrimonia Pilosa Herba</i>	1:256
Stemona root	<i>Stemona Sessilifolia Radix</i>	>1:1	Isatis Root	<i>Isatidis Tinctoria Radix</i>	>1:1
Indigo or Isatis Leaves	<i>Isatis Tinctoria Folium</i>	1:2	Wintermelon Peel	<i>Benicasa Hispida Epicarpium</i>	1:32
Sea weed	<i>Sargassum Pallidum Herba</i>	>1:1	Herba Hedyotis Diffusae	<i>Hedyotis diffusa Willd</i>	1:16
Tremella fruiting body	<i>Tremela Fuciformis Fructificatio</i>	1:2	Coptis Rhizome	<i>Coptis Chinensis Rhizoma</i>	<1:512

would be presented.

Microorganisms and growth condition

The test organisms included the bacteria *H. pylori* ATCC 11639. Stored bacteria were restituted and incubated anaerobically on blood agar plates (Columbia agar base II, Oxoid), supplemented with 10% sheep blood and the following antibiotics: vancomycin (10 mg/l-1), polymyxin B (2500 U/l-1), trimethoprim (5 mg/l-1) and amphotericin B (4 mg/l-1). Plates were incubated for 48 h at 37°C under micro-aerobic conditions. Broth cultures

were grown in Brucella Broth (Difco, Sparks, MD) supplemented with 3% newborn calf serum. Ferric chloride and desferal (deferoxamine mesylate) were purchased from Sigma, filter-sterilized, and used at the indicated concentrations.

Antimicrobial sensitivity broth microdilution method

Sensitive test of bacteria were carried out by broth dilution method on 96 micro-well plate based on recommendations of the (National Committee for Clinical Laboratory Standards, 2000). Minimum

inhibitory concentration (MIC) was determined. The inoculums were prepared by suspending colonies from a 24 h anaerobic sheep blood agar plate in 5 ml of pre-reduced Anaerobe broth to a density equal to that of a no. 1 McFarland standard. The suspension was further diluted to give a final inoculum size of 10⁵ CFU per well (10⁶ CFU/ml). For evaluation by bacterial sensitive test, all kinds of juice of herbs were prepared in serial twofold dilutions in anaerobe broth within a dilution range from 1:1 to 1:512. In each micro-well contained 50 µl grade diluted agents, 50 µl bacterial stain suspension. Final test volumes of 100 µl were dispensed into each well. In final two

ranks, the rank contained herb agent and is only considered as negative control and the rank contained bacteria only is considered as positive control. After 24 h incubation of the micro-well plate in an anaerobic work station (BUG BOX DUAL, Fuskinn, England) in an atmosphere of 80% N₂, 10% H₂ and 10%CO₂ at 37°C, bacterial growth was observed by stereoscope and recorded. At same time, from every micro-well, 50 µl of treated bacterial suspension was removed and smeared on new plates correspondingly for bacterial colony count after incubated another 48 h. At the beginning concentration of no bacteria growth in plates is considered as individual agent minimum inhibitory concentration (MIC) in microdilution correspondingly for individual strain. The tests were carried out by quadruplication and the result was showed by mean.

Synergistic effect of Chinese Herb on *H. pylori*

Chinese herbalism is classified in this study according to traditional Chinese medicine theory. Herbs that "*Clear Heat and Eliminate Toxins*": Herba Hedyotis Diffusae, Patrinia, Ampelopsis Root, Indigo or Isatis Leaves, Honeysuckle Flower, Chinese Wolfberry, Indigo (Isatis) Leaf processed, Houtuynia, Tremella Fruiting Body, Isatis Root, Chinese Wolfberry. Herbs that "*Clear Heat and Dry Dampness*": Coptis Rhizome, Amur Cork Tree Bark. Herbs that "*Cool and Transform Phlegm-Heat*": Gallnut (cocoon) of Chinese Sumac, Schisandra Fruit, Ophiopogonis tuber. Cool, Spicy Herbs that "*Release the Exterior*": Field Mint, winter jasmine flower, Angelica Root, Chrysanthemum Flower. Herbs that "*Expel Parasites*": Borneo camphor etc.

Synergistic group's effects of Chinese Herbs on *H. pylori* were processed according to herb class of traditional Chinese medicine theory and potential of bactericidal activity respectively. Various herbs were mixed proportionally. The methodology of antimicrobial sensitivity tests was similar as above.

Treatment of 45 cases of chronic gastritis in *H. pylori* infected with a prescription based on Chinese herbs, "*Clear Heat and Eliminate Toxins*"

The study was approved by the Ethics Committee of Shanghai 8th Hospital.

General data

The cases were originated from Shanghai 8th Hospital, diagnosed as gastritis according to a modified criterion of national symposium on the diagnosis and treatment of chronic gastritis in October 1982, Chongqing (Li, 1983).

The examinations of gastroscop were made for

confirmation and the chronic gastritis removed become malignant or has becomes malignant in tendency. The biopsies of gastric mucosa were taken from the same site pre- and post-treatmently as possible. A serum antibody test kit was used for evaluation of *H. pylori* eradication. Serum IgG against *H. pylori* was detected by ELISA using *H. pylori* kits produced by Shanghai Jing Ying Biotechnology Company (China).

There were 80 patients, who were divided into two groups randomly. Treated group had 45 cases, 27 were male and 18 female; ranging 29 to 63 years in age, 48.8 ± 13.3 years on average; course of disease 1.4 to 29.0 years, 12.7 ± 9.4 on average. Control groups had 35 cases, 22 were male and 13 female; ranging 31 to 64 years in age, averaging 46.1 ± 12.5 years; course of disease 3.6 to 26.0 years, averaging 10.9 ± 8.8 years. The patients' sex, age, course of disease and disease situation were comparable, with no statistically significant difference between two groups (P>0.05).

Treatment method

Treatment group: Chinese herbs are composed of Houtuynia 30 g, Patrinia 24 g, Official Magnolia 18 g, Coptis Rhizome 15 g, Ophiopogonis tuber 15 g, Gallnut (cocoon) of Chinese Sumac 15 g, etc. The medicine is dried, grinded to a fine powder, sifted over a 60 mesh. 30 g powders were wrapped up in one package and sealed keeping.

Their was taken orally three times a day, 2 packets for each time, 4 weeks as one course of treatment, two courses in general. It was adjusted into the pastel and taken with warm water and honey.

Control groups: Colloidal Bisuth Pectin Ccpsules (Anter Pharmacy, Xian, China) 150 mg/time, 2 to 3 times daily, on an empty stomach, 45 min before meals; Metronidazole Tablets 500 mg / time, 3 times daily, 30 min after meals. 4 weeks as one course of treatment, two courses in general.

RESULTS

Compare study of Chinese herbs on *H. pylori*

It was showed that all except 15 extract from 40 plants showed activity against the microorganism, but the most active extracts were those from Chinese nut-gallare, clove, heartleaf houttuynia herb, hairyvein agrimonia herb, and coptis root. Minimum inhibitory concentrations of five herb juice were within the range of 1:256 to 1:512 (Table 1). The results of synergistic effect of Chinese herbs on *H. pylori* were showed on Table 2. The classes of "*Clear Heat and Eliminate Toxins*" (group 1 and 2) and the class of Coptis Rhizome and Gallnut (cocoon) of Chinese Sumac were revealed the strongest effect.

Table 2. Synergistic effect of Chinese Herb on *H. pylori*.

Chinese herbalism classifies according to traditional Chinese medicine theory	MIC
Clear heat and eliminate toxins (group 1):Indigo (Isatis) leaf processed, Houtuynia,Tremella Fruiting Body, Isatis Root, Chinese Wolfberry.	>1:512
Clear Heat and Eliminate Toxins (group 2): Herba Hedyotis Diffusae, Patrinia, Ampelopsis Root, Indigo or Isatis leaves, Honeysuckle Flower, Chinese Wolfberry	>1:512
Herbs that Clear Heat and Dry Dampness: Coptis Rhizome, Amur Cork Tree Bark	1:128
Herbs that Cool and Transform Phlegn-Heat: Sophora Root, Gallnut (cocoon) of Chinese Sumac, Schisandra Fruit, Ophiopogonis tuber	1:32
Spicy Herbs that Release the Exterior: Field Mint, Winter jasmine flower, Angelica Root, Chrysanthemum Flower.	1:16
Chinese herbalism classifies according to intension of bacterial inhibitory	
Coptis Rhizome, Houtuynia	1:256
Coptis Rhizome, Gallnut (cocoon) of Chinese Sumac	>1:512
Coptis Rhizome, Agrimony	1:128
Houtuynia, Agrimony, Clover Flower Bud, Gallnut (cocoon) of Chinese Sumac	
Houtuynia, Gallnut (cocoon) of Chinese Sumac	1:512
Coptis Rhizome, Houtuynia, Gallnut (cocoon) of Chinese Sumac	1:256
Agrimony, Clover Flower Bud	1:128
Houtuynia, Clover Flower Bud	1:256
Gallnut (cocoon) of Chinese Sumac, Clover Flower Bud	1:256
Coptis Rhizome, Houtuynia, Agrimony, Clover Flower Bud, Gallnut (cocoon) of Chinese Sumac	1:64
Coptis Rhizome, Agrimony, Gallnut (cocoon) of Chinese Sumac	
Houtuynia, Agrimony, Gallnut (cocoon) of Chinese Sumac	1:256
Houtuynia, Clover Flower Bud, Gallnut (cocoon) of Chinese Sumac	1:512
Agrimony, Clover Flower Bud, Schisandra Fruit Gan	1:128
Agrimony, Clover Flower Bud, Schisandra Fruit Gan Houtuynia, Gallnut (cocoon) of Chinese Sumac	1:16
Coptis rhizome, agrimony, clover flower bud, schisandra fruit Gan, Houtuynia, Gallnut (cocoon) of Chinese Sumac	1:8

The therapeutic effects of Chinese herbs

The criteria of therapeutic effect was issued in "The Guidelines for Clinical Investigation of New Traditional Chinese Materia Medica for Treatment of CAG" made by Ministry of Health of China (Ministry of Health China, 1995). Cured: clinical symptoms and histopathological changes of gastric mucosa disappeared completely, negative result was observed for measuring *H.pylori*. Improved: clinical symptoms basically disappeared but excluding histopathological

changes of gastric mucosa, negative result was observed for measuring *H. pylori*. Ineffective: clinical symptoms and histopathological changes of gastric mucosa no obvious improvement, positive result was observed with both two kinds of methods for measuring *H. pylori*.

Among the 45 cases of the treated group, 38 (84.44%) were cured, 5 (11.11%) improved and 2 (4.44%) ineffective, the total effective rate being 95.55%. While in the 35 cases of the control group, 24 (68.57%) were cured, 2 (5.71%) improved and 9 (25.71%) ineffective, the total effective rate being

74.28%. By t-test, the difference between the total effective rate of the two groups was significant, $P < 0.001$. The treatment group was better than that of control group. The initiating time of effect for the treated group was 1 to 4 days, 2.1 days on average; while those of the control group were 3 to 7 days and 5.2 days.

DISCUSSION

At the present time, there is no generally accepted,

safe and effective therapy for *H. pylori* infections. Currently in modern western medicine, the disease is treated by means of depressing the acid, improving the defense ability of the gastric mucosa, and killing *H. pylori* so that the ulcer can be healed. However, the effect is unsatisfying and is not apt to repeat. So a lot of herbal drugs, as well some source plants have been characterized for anti-*H. pylori*. Du P (Du et al., 2001) reported that *Radix scutellariae*, *Flos lonicerae*, *Radix isatidis*, *Indigo naturalis*, *Fructus chebulae*, *Semen ginkgo*, *Cortex phellodendri*, *Rhizoma corydalis* and *Cortex fraxini* have obvious effect of anti-bacterium to *H. pylori*. Gadhi et al. (2001) studied the anti-*H. pylori* effect of the extracts and the fractions obtained from *Aristolochia paucinervis* rhizome and leaves. It was found that only the methanol extracts and the hexane fractions of either the rhizome or the leaves exhibited an inhibitory activity. The leaf hexane fraction APLH demonstrated a higher inhibitory activity (MIC: 4 mg/ml) than the rhizome hexane fraction APRH (MIC: 16 mg/ml), the leaf methanol extract APLM (MIC: 32 mg/ml) and the rhizome methanol extract APRM (MIC: 128 mg/ml). Stamatis et al. (2003) have studied the anti-*H. pylori* effect of 70 Greek plant extracts and a number of commercially available herbs used traditionally in folk medicine against gastric ailments, peptic ulcer included. The extracts of *Anthemis melanolepis*, *Cerastium candidissimum*, *Chamomilla recutita*, *Conyza albida*, *Dittrichia viscosa*, *Origanum vulgare* and *Stachys alopecuros* have been proved active against one standard strain and 15 clinical isolates of *H. pylori*. Li et al. (2005) has reported the anti-*H. pylori* action of 30 Chinese herbal medicines which have been frequently prescribed since ancient times for treating gastritis-like disorders. Among the 30 tested materials, the ethanol extracts of *Abrus cantoniensis* (Fabaceae), *Saussurea lappa* (Asteraceae) and *Eugenia caryophyllata* (Myrtaceae) were strongly inhibitory to all test strains (MICs: approximately 40 mg/ml). The herbs they used were most prescribed gastritis-treating in China. Synergistic effect studies have not been carried out in aforementioned reports.

40 Chinese herbs on *H. pylori* have been carried out in this study. Under the theory of traditional Chinese medicine (TCM), they take on 'settling heat and dispelling humid', detoxification and haemostatic, 'depressing heat of body and promoting several positive state' and so on (Li and He, 1998). Traditional, they have been variedly used for treating inflammation and detumescencing in Chinese folklore medicine. But no report has been found about the pharmacology comparative study of these herbs on *H. pylori*.

The experimental results showed that all except 15 extract from 40 plants were demonstrated to possess higher anti *H. pylori* activity. The most active extracts were Gallnut (cocoon) of Chinese Sumac (MIC: <1:512), Coptis Rhizome (MIC: <1:512), Houtuynia (MIC: 1:512), Clover Flower Bud (MIC: 1:256) and Agrimony (MIC: 1:256).

Chinese herbs were classified according to "the Four

Energies", "the Five Flavors", "the Four Directions" and their relationship to "the 12 Internal Organs" in TCM. "The Four Energies" are cold, cool, warm and hot. Herbs that have a "cold energy" are used to treat inflammatory and toxic conditions. Some of these are Lonicera and Forsythia blossoms and gentian root for instances. The effect of Chinese Herb is co-operation of multiple assorted agents. Grouped by herb class of TCM theory and potential of bactericidal activity respectively, some effective herb was put together to observe its synergistic effect on *H. pylori*. The classes of "Clear Heat and Eliminate Toxins" (group 1 and 2) and the combination of Coptis Rhizome and Gallnut (cocoon) of Chinese Sumac were revealed the strongest effect on *H. pylori*.

A prescription contained mainly herbs of "Clear Heat and Eliminate Toxins" is prepared through clinical screening and proved to have good effects in treating chronic gastritis with *H. pylori* infection. Among the 45 cases of the treated group, 38 (84.44%) were cured, 5 (11.11%) improved and 2 (4.44%) ineffective, the total effective rate being 95.55%. While in the 35 cases of the control group, 24 (68.57%) were cured, 2 (5.71%) improved and 9 (25.71%) ineffective, the total effective rate being 74.28%. By t-test, the difference between the total effective rate of the two groups was significant, $P < 0.001$. The effect of the treated group was obviously better than that of the control group. The clinical symptoms and histopathological changes of gastric mucosa were disappeared completely. No any serious adverse effects were found during treatment.

Conclusions

Therefore, the prescription contained herbs of "Clear Heat and Eliminate Toxins" is an important drug in treating chronic gastritis caused by *H. pylori*. The *in vivo* data confirmed the potential usefulness of these plants for treatment and prevention of *H. pylori* disease.

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