

***In vitro* Susceptibility of *Helicobacter pylori* to Licorice Extract**

Masoomeh Malek Jafarian and Kiarash Ghazvini*

Department of Microbiology and Virology, Mashhad University of Medical Sciences, Mashhad, Iran.

Abstract

Helicobacter pylori is the causative agent of gastritis and peptic ulcer disease. Eradication of *H. pylori* is hard to achieve and often require multiple antibiotics regimens. These regimens are quite expensive and have substantial side effects. Licorice is a traditional drug which is frequently used for gastric discomforts in Iran. The susceptibility of *H. pylori* to the extract of licorice has been investigated in the present study.

One hundred and eighty clinical *H. pylori* strains were isolated in this study. For comparison and control, *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* were also included in this study. Agar dilution assay was used to test the susceptibility of the clinical isolates of *H. pylori* to the *Glycyrrhiza* extract at different concentration. The assay plates had 25, 50, 100, 150, 200, 300 and 400 mg/ml of licorice extract.

Licorice extract inhibited the *H. pylori* strains with a MIC range of 50-400 mg/ml. The present results show that therapeutically administered concentrations of licorice extract could have growth-inhibiting effect on *H. pylori in vitro*.

The above preliminary experiments indicated strongly that licorice extract has some anti-*H. pylori* property and *H. pylori* shows susceptibility to licorice extract in concentration ranges that are achievable in the stomach. The resistance of other bacterial species indicates the possibility of selective therapy without adverse effects on the normal bacterial flora.

Keywords: *Helicobacter pylori*; Peptic ulcer; Licorice; Agar dilution assay.

Introduction

Helicobacter pylori is the causative agent of gastritis and peptic ulcer disease and is probably related to gastric cancers (1, 2, 3, 4). In industrialized countries, the incidence of infection is about 0.5%-1.0% per year and approximately 50% of 60-year-old adults are infected (5). In non-industrialized countries, the prevalence rates are considerably higher, and even children are commonly infected (5). Thus,

it is likely that one-half of the world's population is infected with *H. pylori* (5).

Because the mode of transmission is unknown, effective preventative measures are lacking (6). Likewise, eradication of *H. pylori* is hard to achieve and often requires complex regimens (3, 7). These regimens are quite expensive and have substantial side effects, in particular the emergence of resistance to antibiotics (3). Therefore, it is recommended to limit antimicrobial eradication treatment to symptomatic patients with duodenal and gastric ulcers (3). However, if it is accepted that *H. pylori* infection results in a sequence of changes

* Corresponding author:

E-mail: kiarash_ghazvini@yahoo.com

in the gastric mucosa that could ultimately lead to intestinal metaplasia and gastric cancer (8), the indication for treatment will extend to all infected individuals (9). The classic antibiotics are not suitable for such a widespread use and alternative *H. pylori* eradication drugs which would help in eradication of *H. pylori* would be appreciated.

Licorice is the name applied to the roots and rhizomes of *Glycyrrhiza* species and has been used for medicinal purposes for at least 4000 years (10). Licorice is one of the most frequently used natural medicines (10). Anti-*H. pylori* activities of extractum liquiritiae, glycyrrhizin and its metabolites have been reported in some other studies (11, 12, 13). In the present study the susceptibility of *H. pylori* to the extract from the *G. glabra* has been investigated among 180 isolates of *H. pylori*.

Experimental

Methods

One hundred and eighty clinical *H. pylori* strains were isolated from gastric biopsies in this study. Specimens were incubated microaerobically on Colombia agar supplemented with 7% horse serum, 5% sheep blood at 37°C for 4 days in GasPak jars. After identification, colonies were suspended in Brucella broth to give the turbidity equivalent to $OD_{570} = 0.23$; this resulted in suspensions containing about 1×10^7 colony forming units (cfu)/ml. Final concentration of the bacteria used was 1×10^5 cfu/ml. For comparison and control, *Staphylococcus aureus* PTCC 1112, *Escherichia coli* PTCC 1338, *Pseudomonas aeruginosa* PTCC 1074 were also included in this study. Suspension of *S. aureus*, *E. coli*, and *P. aeruginosa* were prepared. Inoculum suspensions of these bacteria were adjusted to a turbidity corresponding to similar concentration of *H. pylori*.

Agar dilution assay was used to test the susceptibility of the 180 clinical isolates of *H. pylori* to the *Glycyrrhiza* extract at different concentration, as recommended by the National Committee for Clinical Laboratory Standards (NCCLS).

The roots of *Glycyrrhiza glabra* (500 g) were extracted with 2 L of methanol for 2 days each time. The methanol solutions were combined and then evaporated in vacuum to yield 97 g of residue. The *Glycyrrhiza* extract was neutralized and then serial dilutions of the drugs were prepared in plates according to the standard procedure. After solidification, the plates were incubated at 37°C for 2 h in order to dry the agar surface. The assay plates were estimated to have 25, 50, 100, 150, 200, 300 and 400 mg/ml of active licorice extract. Inoculum suspensions were spotted on the agar. Growth controls were performed on agar containing physiological saline instead of drug dilution. Incubation of the inoculated plates was performed under the same culture conditions as used for inoculum preparation. Growth inhibition effect was observed after 5 days of incubation. Minimum inhibitory concentration (MIC) was recorded by reading the lowest drug concentration that inhibited visible growth. The MIC was presented in the range of MIC for susceptible organisms.

Results and Discussion

The drug shows an antibacterial effect and the MICs of licorice extract against *H. pylori* were in the range of 50-400 mg/ml. In the control strains, (*S. aureus*) the extracts had MICs of about 200 mg/ml and the control strains of *E. coli* and *P. aeruginosa* were not inhibited up to a concentration of 400 mg/ml.

Licorice extract inhibited the *H. pylori* strains with a MIC range of 50-400 mg/ml. The MIC_{50} is less than 100 mg/ml and MIC_{90} is about 300

Table 1. Frequency and percent of sensitive and resistant *H. pylori* to different concentrations of Licorice extract.

Drug concentrations	25 mg/ml	50 mg/ml	100 mg/ml	150 mg/ml	200 mg/ml	300 mg/ml	400 mg/ml
Bacterial growth	176/180 (97.7%)	137/180 (76.1%)	79/180 (43.8%)	60/180 (33.3%)	47/180 (26.1%)	16/180 (8.8%)	0/180 (0%)
Bacterial Inhibition	4/180 (2.2%)	43/180 (23.8%)	101/180 (56.1%)	120/180 (66.6%)	133/180 (73.8%)	164/180 (91.1%)	180/180 (100%)

mg/ml. No strain in this study showed resistance to the licorice. The present results show that therapeutically administered concentrations of licorice extract could have growth-inhibiting effect on *H. pylori in vitro*. Thus, licorice extract may be suitable for *H. pylori* eradication and in this way prevent gastro-duodenal ulcer relapse.

Similar result has been reported by other researchers although some studies have used purified fractions of licorice and reported more potent anti-helicobacter activity (11, 12, 13). The extract investigated in this study had weaker anti-*H. pylori* activity in comparison to other studies; however, since we intend to study antibacterial effect of licorice as a crude drug, and we did not purified and isolate the different fraction of extract, this result is somehow expected (14, 15).

In Asia, Licorice has been one of the most frequently used natural medicines for at least 4000 years (12, 13, 16). Licorice is frequently used in Iran as traditional drugs for gastric discomforts and has also been recommended for its detoxification effect (17). The above preliminary experiments indicated strongly that licorice extract has good anti-*H. pylori* activities, and this effect of licorice has also been reported by other researchers (14, 15). As demonstrated in this study and mentioned also by other studies, the antibacterial spectrum of licorice is obviously confined to *H. pylori* and probably staphylococci (15). In contrast to the antibiotics used at present in the *H. pylori* regimens, it has the benefit of leaving the normal bacterial flora of the body unchanged and not selecting resistant bacteria.

In addition, although drug resistance is an increasing problem in many infectious diseases during recent years, it is interesting that licorice flavonoids exhibited anti-*H. pylori* activity against not only antibiotic sensitive strains but also antibiotic resistant strains (13, 18). Also in our study in spite of long history of licorice usage as a crude drug in Iran (17), no isolated strains have developed resistance to the licorice extracts.

Licorice is chemically inert, non-absorbable and free of severe side effects (19). Licorice is therapeutically administered by the oral route and gastric concentrations higher than the MICs may be attained by oral administration of licorice

extract. Therefore, the drug could be considered as an alternative to the more aggressive regimens for *H. pylori* eradication therapy, and should be evaluated in clinical studies. Thus, there is a real chance that a therapeutic regimen for the eradication of *H. pylori* can be found that is not confined to symptomatic patients with duodenal and gastric ulcers but also includes treatment of non-ulcer dyspepsia and asymptomatic *H. pylori* infections.

Many flavonoids with one or more isoprenoid groups have been isolated from *Glycyrrhiza* species which could have anti-*H. pylori* activity (10, 11, 20). Very recently, it has been reported that dimethylsiloxane (a compound related to licorice extract), has a therapeutic effect in treating gastric ulcers comparable to that of the H₂-blocking agent as ranitidine and *H. pylori* was eliminated more effectively in patients in the dimethylsiloxane group than in the control group (21). These compounds may be useful as lead compounds in the development of a new class of anti-*H. pylori* agents.

In conclusion, *H. pylori* shows susceptibility to licorice extract in concentration ranges that are achievable in the stomach under therapeutic conditions. The resistance of representatives of other bacterial species indicates specific interactions of the drugs with *H. pylori*, and offers the possibility of selective therapy without adverse effects on the normal bacterial flora. In view of the pharmacological data, licorice extract appears to be a promising agent for the treatment of all forms of *H. pylori* infection. Especially if we consider some other advantages of licorice as nontoxic even in high dosages so applicable to young children and very inexpensive so as not to be restricted to rich people or industrial countries, it should be considered as a real chance for treatment of *H. pylori* infection. Despite all these findings, licorice could only become a practical measure for treating or preventing *H. pylori* infections after complete clinical studies.

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