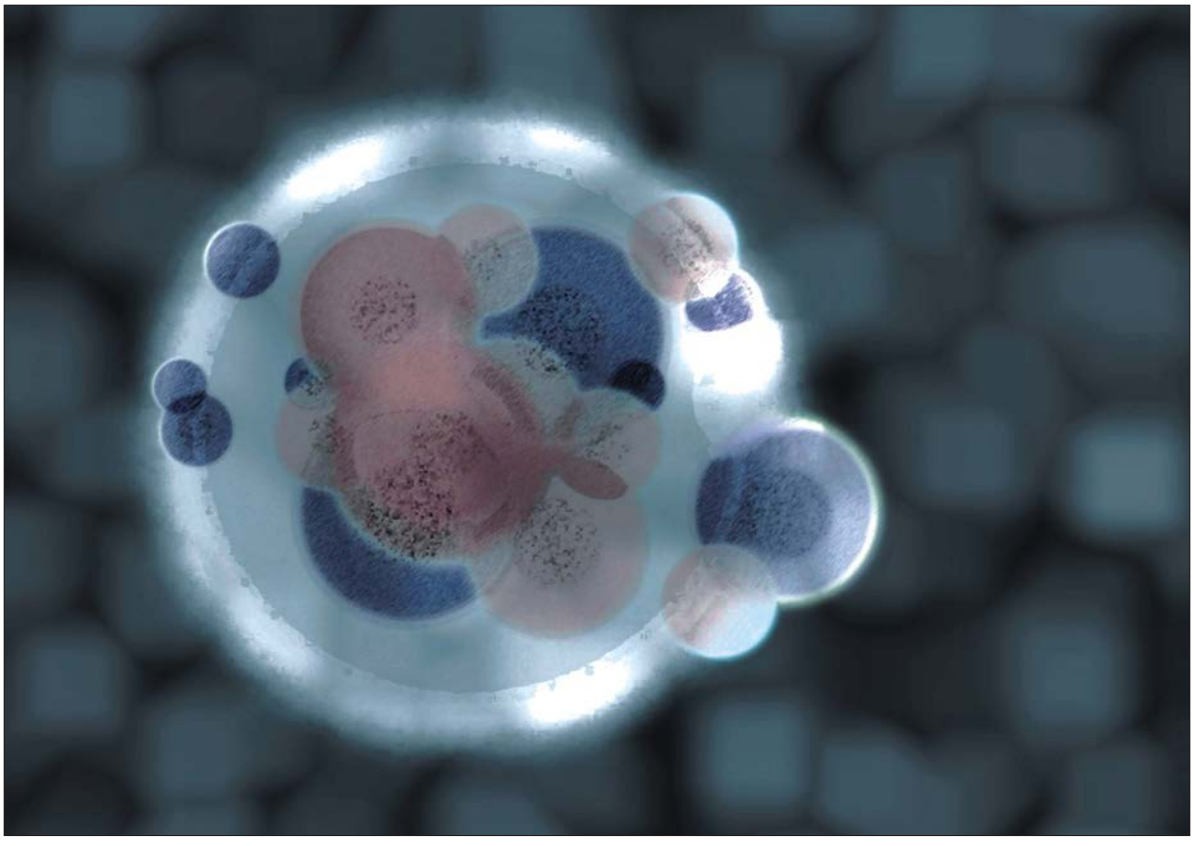

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An Evaluation of Antibacterial Activity of *Glycyrrhiza glabra* Extract on the Growth of *Salmonella*, *Shigella* and ETEC *E. coli*

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Abstract: In the present study, the *in vitro* inhibitory effects of *G. glabra* extract against the growth of *Salmonella typhi*, *S. paratyphi B*, *Shigella sonnei*, *S. flexneri* and enterotoxigenic *E. coli* (ETEC *E. coli*) was investigated using well and disc diffusion method. *Shigella* spp. and enterotoxigenic *E. coli* but *Salmonella paratyphi*B showed no susceptibility to liquorice with concentrations lower than 7.5%, however all tested bacterial strains exhibited susceptibility to high concentration of liquorice. Results obtained from present study showed that *G. glabra* can be considered as an alternative herbal antibacterial agent against the bacterial strains tested.

Key words: *Glycyrrhiza glabra*, liquorice, antibacterial activity

INTRODUCTION

Herbal traditions have been passed down and refined with scientific understanding, providing information to assist in health maintenance. Approximately 25% of all prescription drugs are derived from trees, shrubs, or herbs. Their increasing use in recent years is an evidence of a public interest in having alternatives to conventional medicine. Liquorice comes from the Greek word glykyrrhiza, literally meaning sweet root, referring to the root of a small plant of the pea family. Licorice, the root and rhizome of the *Glycyrrhiza* spp. including *Glycyrrhiza* uralensis (Hatano *et al*., 2000; Shibata, 2000), *Glycyrrhiza glabra* (Mitscher *et al*., 1980; Okada *et al*., 1989; Shibata, 2000; Fukui *et al*., 1988; Demizu *et al*., 1988) and *Glycyrrhiza inflata* (Haraguchi *et al*., 1998a; Kajiyama *et al*., 1992; Okada *et al*., 1989; Shibata, 2000), is currently used in pharmaceutical, tobacco and food industries. It has been used for centuries as an herbal therapeutic substance because of its wide-ranging therapeutic properties, including relief of rheumatic and other painful illnesses and its healing effects on the gastric ulcers (Shibata, 2000).

Licorice extracts obtained from *G. uralensis*, *G. glabra* and other *Glycyrrhiza* species have been used as a drug for gastritis, gastric and duodenal ulcers, stomachic, cough medicine and also as a chemo-preventive agent in Japan. Licorice (*G. glabra*) and its extract have been used as bronchial, gastrointestinal, liver, bile and urological remedies in the western countries (Willuhn, 1994). Licorice is also used for treatments of gastritis, ulcer, hemorrhoids, melanoma and food poisoning, in China (Zheng and Guo, 1993).

Recently, various biological functions of Licorice including anti-protozoal (Chen *et al*., 2001), anti-inflammatory (Shibata, 2000), anti-tumor promoting (Shibata, 2000), anti-oxidative (Haraguchi *et al*., 1998b; Okada *et al*., 1989) and antimicrobial (Haraguchiet *et al*., 1998b; Okada *et al*., 1989) activities were reported.

In the present study *in vitro* inhibitory effects of liquorice on the growth of the variety of medically important enteric bacteria including *Salmonella typhi, S. paratyphi B*, *Shigella sonnei*, *S. flexneri* and ETEC *E. coli* was investigated.

MATERIALS AND METHODS

Liquorice and bacterial strains: Reference microbial strains including *Salmonella typhi* and S. paratyphi B, *S. sonnei*, *S. flexneri* and ETEC *E. coli* were obtained from Iranian Scientific and Industrial Research Centre (ISIRC) in lyophilized form.

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The liquorice was obtained from local groceries in the form of powder. Four concentrations of liquorice (5, 7.5, 10 and 15%) were prepared in sterilized normal saline. Agar-well and disc diffusion methods were used to study the inhibitory effect of different concentrations of liquorice against bacterial strains tested (Bauer *et al*., 1966). Muller Hinton agar (Difco, 225250) was used for agar diffusion tests. Growth-inhibitory zone diameters were measured within 24-48h after incubation. Zone diameters (in millimeters) of <5, ≥ 5< 10 and ≥ 10 were defined weak, moderate and good inhibitory effect, respectively.

RESULTS

As shown in Table 1, *in vitro* inhibitory effects of liquorice against the growth of *Salmonella typhi* and ETEC *E. coli* was seen at equivalent or higher concentrations of 7.5%. However *S. paratyphi* B was also susceptible to concentration of 5% liquorice. Liquorice exhibited a poor effect on the growth of *Shigella*. Overall, the increased inhibitory activity of liquorice against all of tested bacterial isolates was observed with higher concentrations of 7.5%. Disc diffusion method showed the same results.

Table 1: Inhibitory effects of different concentratios of liquorice against the growth of *Salmonella typhi, S. paratyphi B, S. sonnei, S. flexneri* and ETEC *E. coli*

	liquorice concentrations (%)			
Bacterial strains	5	7.5	10	15
S. typhi	-*	+	++	+++
S. paratyphi B	+	++	+++	+++
S. sonnei	-	+	+	++
S. flexneri	-	+	+	++
ETEC *E. coli*	-	+	++	+++

*-: No inhibitory effect; +: Weak inhibitory effect; ++: Moderate inhibitory effect; +++: Good inhibitory effect. Zone diameters (mm) of <5, ≥ 5< 10 and ≥ 10 were defined weak, moderate and good inhibitory effect, respectively

DISCUSSION

Antiviral (Lin, 2003; Sasaki *et al*., 2002), antibacterial (Tanaka *et al*., 2001; Friis-Moller *et al*., 2002; Fukai *et al*., 2002a; Tsukiyama *et al*., 2002; Krausse *et al*., 2004) and anti parasitic (Chen *et al*., 1993) activities of liquorice or it's components have been documented. Lin (2003) reported that glycyrrhizic acid (GL), a component of licorice root (*Glycyrrhiza radix*), was active against EBV replication in super infected Raji cells (Lin, 2003). Sasaki *et al.* (2002) demonstrated that glycyrrhizin had the potential activity against NSI-HIV replication in peripheral blood mononuclear cell cultures by inducing the production of beta-chemokines (Sasaki *et al*., 2002). Haraguchi *et al.* (1998a) reported that Licochalcone A-D and echinatin, retrochalcones, isolated from the roots of *Glycyrrhiza inflata*, showed antimicrobial activity. Krausse *et al.* (2004) and Fauki *et al.* (2002b) investigated *In vitro* activity of some herbal extracts including glycyrrhizic acid (GL) on the growth of 29 *Helicobacter pylori* strains. They showed that GL was a potent compound. In another study Licochalcone A, extracted and purified from Chinese licorice roots, showed *in vitro* inhibitory effect on the human pathogenic *Mycobacterial* and *Legionella* species (Friis-Moller *et al*., 2002). Tanaka *et al.* (2001) measured the antibacterial activity of compounds obtained from licorice against the bacterial strains including *Streptococcus pyogenes*, *Haemophilus influenzae* and *Moraxella catarrhalis* isolated from upper airway respiratory tract. Among the tested compounds, licoricidin exhibited the highest activity against all tested microorganisms Fukai *et al.* (2002b) demonstrated antimicrobial activity of flavonoids isolated from licorice against methicillin sensitive *Staphylococcus aureus* (strains FDA 209P and Smith), methicillin resistant *S. aureus* (strains K3 and ST 28), *Micrococcus luteus* ATCC 9341 and *Bacillus subtilis* PCI 219(MICs3.13-25 mg mL^{-1}) but not against *Klebsiella pneumoniae PCI 602* and Pseudomonas aeruginosa IFO 3445 (Fukai *et al*., 2002b). Finally, in a study coducted by Hwang *et al.* (2004), the methanol extracts of five tropical plants including *Glycyrrhiza glabra* exhibited potent antibacterial activity against the cariogenic bacterium *Streptococcus mutans* (Hwang *et al*., 2004). However, there is little published data about its activities against gram negative bacilli.

In our study bacterial growth of all strains tested was inhibited by liquorice at concentrations higher than 7.5% in both well and disc diffusion methods. Although liquorice exhibited a poor effect on the growth of *Shigella*, it could be improved by use of higher concentrations of the prepared liquorice. Finally, the antimicrobial properties of liquorice and the fact that liquorice is naturally occurring and nontoxic to humans make it a promising biological alternative to chemical preservatives and disinfectants and especially it might be of interest as a new class of antibacterial drug in the treatment of infections caused by the mentioned microorganisms.

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