Antibacterial Effects of *Galla Rhois* Extract against *Streptococcus suis* Infection in Mice

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**ABSTRACT** - *Streptococcus suis* (*S. suis*) is a major swine pathogen and an emerging zoonotic agent and is an increasing public health problem across Asia. The present study was undertaken to estimate the antibacterial effect of GR extract and therapeutic effect of GR extract against *S. suis* infection in mice. At the concentration of GR extract 2.5 mg/ml, the antibacterial effect was not shown on *S. suis*. However, the antibacterial effect against *S. suis* was observed at the concentration of GR extract 5.0 mg/ml. Oral administration of GR extract at the dose of 10 mg/kg showed a therapeutic effect for *S. suis* infected BALB/c mice. The mortality of GR extract-treated mice at the concentration of 5, 10 and 20 mg/kg was 80%, 70%, and 50% at 12 days, respectively, while that of untreated mice was 100% at 8 days after a lethal dose of *S. suis* infection. The results of our study strongly indicate that GR extract has potential as an effective for *S. suis* infection in mice.

**Key words:** *Galla rhois*, methanol extract, *Streptococcus suis*, antibacterial effect, mouse

**Introduction**

*Streptococcus suis* (*S. suis*) is a gram-positive bacterium distributed worldwide that causes meningitis, endocarditis, septicemia, septic arthritis, pneumonia, and abortion in pigs and humans¹. In intensive swine industry, *S. suis* infection is causing considerable economic losses and animal health care problems for the pig farming industry worldwide². The natural habitat of *S. suis* is the upper respiratory tract and the intestinal tract³,⁴. In adult pigs, carriage of *S. suis* is usually asymptomatic but colonized sows can infect their piglets after nasal or oral contact⁵. Newborn pigs can also become infected during parturition when they contact, swallow or aspirate *S. suis* from sow vaginal secretions⁶. In young pigs, *S. suis* infection causes a wide variety of diseases, including meningitis, septicemia which are the main causes of mortality⁷. As an emerging zoonotic pathogen, *S. suis* can be transmitted to humans by direct contact⁸-¹⁰. *S. suis* is also emerging as a serious zoonotic pathogen of humans particularly in South East and East Asia where it is one of the most common causes of human meningitis¹¹,¹². In 2005, a large outbreak of 215 cases *S. suis* infections occurred in Sichuan, China, resulting in 38 deaths¹³. *S. suis* from pigs was thought to be the origin of the outbreak.

At present, prevention and control of the disease are based on the use of the autogenous vaccine and antibiotics¹⁴. Prior to the emergence of *S. suis* strains, penicillin was the original choice of treatment¹⁵. Then, macrolides and fluoroquinolones, such as erythromycin, tylosin and enrofloxacin, were considered as the effective substitutes for penicillin because *S. suis* isolates are historically highly susceptible to these drugs¹⁶. However, some of the commonly used antibiotics such as erythromycin and enrofloxacin for treatment and prevention of *S. suis* infections in pigs are becoming less efficient, due to an increase in resistance among *S. suis* isolates in recent years¹⁷-¹⁹.

Conventional herbal medicines have long been used as remedies against infectious diseases in Asian countries including Korea and China, and may be an alternative treatment to *S. suis* infections¹⁸,²⁰. *Galla Rhois* (GR) has long been used in traditional Asian medicine to treat diarrhea, persistent coughing and spontaneous perspiration in man.
because this product has antidiarrheic, astringent and hemostatic properties. GR is a harmless natural material that contains a number of tannin-derived components, including methyl gallate and gallic acid. Gallotannins are a class of hydrolysable tannin polymers formed from gallic acid, which has antifungal and antiviral properties. Methyl gallate and gallic acid have a recognized growth-inhibiting activity against Escherichia coli and Streptococcus spp. and does not negatively influence the growth of lactic acid-producing bacteria.

However, the antibacterial activities of GR against S. suis is unknown. In this study, the antibacterial effects of GR extracts against S. suis were investigated in vitro and in vivo.

Materials and Methods

Bacteria and culture
S. suis (KVCC-BA0700673)) was obtained from the Korean Veterinary Culture Collection (Anyang, Korea). The cultures were incubated in brain heart infusion (BHI) broth (Difco Laboratories, Detroit, USA) for 18 hours at 37°C. The stock organism was stored in BHI broth containing 50% glycerol at −80°C for later use.

Preparation of GR extracts
GR powder was obtained from GS Bio (Jeonju, Korea), who produced the powder from dried plant material and analyzed its components as previously described. Briefly, one kg of plant material dried in an oven at 60°C for three days was twice extracted with methanol at room temperature, the residue was removed by filtration (Toyo filter paper no. 2, Toyo Roshi Kaisha, Ltd., Tokyo, Japan) and the filtrate was concentrated using vacuum rotary evaporation (Iwai Co., Tokyo, Japan) followed by freezing dry to powder. The GS extracted powder was suspended into a 1,000 mg/ml stock solution. The stock solution was then used to prepare various test solutions by diluting with distilled water. The composition of the crude extracted powder was analyzed using chromatography on a silica gel column (70-230 mesh; Merck, Darmstadt, Germany) and fractionation on a preparatory high-performance liquid chromatography column (Delta Prep 4000, Waters, Ontario, Canada). Tannins account for 45.8% of the total composition of GR, and methyl gallate and gallic acid comprised 16.4% and 4.3% of that, respectively.

Determination of antibacterial activity
Bacteria were diluted with phosphate-buffered saline (PBS) solution (pH 7.4) to $2 \times 10^8$ CFU/ml, added in different concentrations (1.0, 2.0, 5.0 mg/ml) of GR extract, and incubated at 37°C for 0, 2, 4, and 8h. After incubation and proper dilution, 1.0 ml of each solution was plated onto agar medium supplemented with 2% (v/v) newborn bovine serum (Difco Laboratories, Detroit, USA) to assess bacterial colony-forming units (CFUs).

Animal challenge test
Forty 6-week-old female BALB/c mice (average body weight, 20.7 ± 0.6 g) purchased from Samtako Bio Korea Co. Ltd. (Osan, Korea) and were acclimated for one week. All mice were randomly divided into four groups (10 mice per group). The experimental groups were treated orally three times per day for 14 days with the following solutions: control group, sterile saline; group 1, GR extract 5 mg/kg body weight; group 2, GR extract 10 mg/kg body weight; group 3, GR extract 20 mg/kg body weight. The mice were housed in a room at a temperature of 22 ± 3°C, a relative humidity of 40-70%, and a 12-h-light/12-h-dark cycle with free access to mouse chow and water. All animal procedures were approved by the Institutional Animal Care and Use Committee of Gyeongsang National University (GNU-LA-2013-18) in Korea. On the day of the experiment, all mice were challenged with a lethal dose of $2 \times 10^9$ CFU of S. suis in 0.1 ml phosphate buffered saline (PBS) with intraperitoneal injection. After bacterial infection, the control group was orally treated with 0.1 ml of sterile PBS and the treated groups were orally treated with GR extract 5, 10 and 20 mg/kg body weight every 24 h during 12 days, respectively. All mice were observed for 12 days for morbidity and mortality.

Statistical analyses
The data were analyzed by a one-way analysis of variance (ANOVA), followed by Student’s t-test. The results are expressed as mean ± SD. A mean difference was significant at the 0.05 level.

Results and Discussion

Determination of antibacterial activity
Antibacterial effect of GR extract against S. suis is presented in Fig. 1. At 2h after incubation, inhibition of S. suis growth in the group treated with 5.0 mg/ml GR extract was significantly lower than that of control (no treatment) ($p < 0.001$). At 4 h and 8 h after incubation, inhibition of S. suis growth in the group treated with 2.0 and 5.0 mg/ml GR extract was significantly lower than that of control ($p < 0.001$), but the number of bacterial cells in the group treated with 1.0 mg/ml GR extract was increased depending on the incubation time. At the concentration of 5.0 mg/ml, the bacteriocidal effect of GR extract was observed on S. suis. Ahn et al. reported that GR-derived tannins (methyl gallate and gallic acid) at the concentration of 10 mg/disc inhibited