Evaluation of Antibacterial Effects of a Combination of Coptidis Rhizoma, Lonicerae Flos, Paeonia Japonica Extracts, and Dioctahedral Smectite Against Salmonella Typhimurium in Murine Salmonellosis

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ABSTRACT - The present study was undertaken to estimate the antibacterial effect of a combination of C. rhizoma, L. Flos, and P. japonica (1:1:1) extracts (CLPS1000) and a combination of the herbal extract mixture and dioctahedral smectite (CLPS1000) against murine salmonellosis. At the concentration of CLPS1000 and CLPS1000 0.5 mg/ml, the antibacterial effect was not observed on Salmonella typhimurium (S. typhimurium). On the other hand, the antibacterial effect against S. typhimurium was observed at the concentration of CLPS1000 and CLPS1000 1.0 mg/ml. Oral administration of Smectite, CLPS1000, and CLPS1000 at the dose of 10 mg/ml showed a therapeutic effect for S. typhimurium infected BALB/c mice. The mortality of Smectite, CLPS1000 and CLPS1000-treated mice was 90%, 90%, and 70% at 12 days, respectively, while that of untreated mice was 100% at 9 days after a lethal dose of S. typhimurium infection. The results of our study strongly indicate that CLPS1000 has potential as an effective of salmonellosis.

Key words: Coptidis rhizoma, Lonicerae Flos, Paeonia japonica, dioctahedral smectite, Salmonella typhimurium

Salmonella extensively causes various disease syndromes, such as self-limiting enteritis, fatal infection in animals, food-borne infection, and typhoid fever in humans.

Generally, antimicrobial agents are used both therapeutically and prophylactically in salmonellosis. However, the increased resistance to these drugs is an unavoidably side effect of antibiotic use, and recent studies have shown a rapid increase in the prevalence of antibiotic resistant Salmonella.

To solve the problem of antibiotic resistance, medicinal herbs and minerals products are attracting considerable attention by many researchers. Many of medicinal herbs are often used in combination to increase their effects. As these herbs contain bioactive components, they have been used many potential clinical and therapeutic applications.

Coptidis rhizoma (C. rhizoma) has been used in oriental medicine as an antibacterial and anti-inflammatory agent.

The extract of C. rhizoma contains a high level of berberine and antibacterial activities in a variety of pathogenic microorganisms, including Salmonella, Pneumococcus, Mycobacterium tuberculosis, Staphylococcus, and Lonicerae Flos (L. Flos) is a widely used herb prescribed in many Korean formulas. It has antibacterial activity, antipyretic, detoxicant, and anti-inflammatory action.

The aqueous extract of Paeonia japonica (P. japonica) has been used in oriental medicine to treat various illnesses because of possession various pharmacological properties such as sedative, analgesic, anti-inflammatory, antimicrobial, immunoaugmentative and anti-stress action.

Medicinal and therapeutic use of mineral products has impacted human health for thousands of years. The intentional consumption of clay materials by humans and animals is known as geophagy, a complex behavior, cosmetics, dietary or nutritional needs and medicinal benefits. Some of clay mineral products have been reported to have anti-diarrheal and antibacterial activity.

Although previous studies had been investigated antibacterial and anti-diarrheal effects for each of Korean traditional herbs and clay minerals, there is little study to investigate...
for the synergistic effect of the combination.

The present study evaluated the therapeutic potentials of the combination of diocathedral smectite (Smectite) and methanol extract from the medicinal herbs, C. rhizoma, L. Flos, and P. japonica for their antibiotic properties against Salmonella typhimurium (S. typhimurium) in vitro and in vivo.

Materials and Methods

Preparation of a combination

C. rhizoma, L. Flos, and P. japonica were purchased from the Korea National Animal Bio Resource Bank (Gyeongnam, Korea). The combination of Korean traditional herbs was referred to Kim40. C. rhizoma, L. Flos, and P. japonica were air-dried in a dark room and ground to a powder. Approximately 100 g of the combination of C. rhizoma, L. Flos, and P. japonica (1:1) powder was soaked in 400 ml of methanol for 24 h under mantle-reflux. The solvent was removed under reduced pressure in a rotary evaporator (N-1000 S, EYELA, Japan). The extracts were filtered using Whatman No.1 filter paper, and the filtrates were evaporated to dryness in a steady air current. The extracts and the combination of herbal extracts and smectite were designated CLP1000 and CLPS1000, respectively. The extracts and the combination were dissolved in distilled water, adjusted to 100 mg/ml final concentration, and sterilized by passage through a Corning syringe filter (0.20 μm, Japan), respectively41.

Bacterial culture and media

Salmonella enterica serovar Typhimurium (S. typhimurium) ATCC 14028 cells were maintained as frozen glycerol stock and cultured in Luria-Bertani (LB) broth or LB broth containing 1.5% agar. Bacteria were grown at 37°C with vigorous shaking to a stationary phase in LB broth.

Determination of antibacterial activity

Bacteria were diluted with phosphate-buffered saline (PBS) solution (pH 7.4) to 2 × 10^4 CFU/ml, added to different concentrations (0.5, 1.0 mg/ml) of CLP1000 and CLPS1000, respectively, and incubated at 37°C for 0, 2, 4, and 8h. After incubation and proper dilution, 100 ml of each solution was plated onto LB agar to assess bacterial colony forming units (CFUs).

Smectite, CLP1000, and CLPS1000 treatment for murine salmonellosis

Specific pathogen free (SPF) female BALB/c mice (Orient Bio, Seoul, Korea) aged 6-8 weeks, weighing 27.1 ± 3.8 g each, were used in this study. All mice were kept at 23 ± 1°C with a 12-h light/dark cycle. They had free access to water and diet and were acclimatized for at least 2 weeks before starting the experiments. Four groups of 10 mice each were used for bacterial infection. Mice were infected intraperitoneally with 2 × 10^6 CFUs of S. typhimurium. After bacterial infection, three groups of mice were orally treated with 0.1 ml of sterile PBS, Smectite (10 mg/ml), CLP1000 (10 mg/ml), and CLPS1000 (10 mg/ml) every 24 h during 12 days, respectively. Infected mice were examined for the viability every 24 h. All procedures described were reviewed and approved by the Animal Ethical Committee of Gyeongsang National University (GNU-LA-15).

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 CLP1000 and CLPS1000 against Salmonella enteric serovar Typhimurium (S. typhimurium) is presented in Fig. 1. At 2 h after incubation, inhibition of S. typhimurium growth in the all treated groups was significantly lower than that of control (no treatment) (p < 0.05). At 4 h and 8 h after incubation, inhibition of S. typhimurium growth in the all treated groups was significantly lower than that of control (no treatment) (p < 0.001), but the number of bacterial cells in the CLP1000 and CLPS1000 group treated with 0.5 mg/ml was increased.

Fig. 1. Antibacterial effect of a combination of C. rhizoma, L. Flos, and P. japonica extracts (CLP1000) and a combination of the extract mixture and diocathedral smectite (CLPS1000) against S. typhimurium. CLP1000 diluted with PBS was used at the concentration with 0 ( ■ ), 0.5 ( ● ) and 1.0 ( ◀ ) mg/ml, and CLPS1000 was used at the dose of 0.5 ( ▲ ) and 1.0 ( ◇ ) mg/ml. Bacterial viability was measured based on CFUs on culture plates for three independent experiments. *Significantly different from the control (p < 0.05). **Significantly different from the control (p < 0.001).
result of mortality rate was depended on experimental conditions.

In conclusion, our results demonstrate that Smectite, CLP1000, and CLPS1000 at the concentration of 10 mg/ml takes effect against S. typhimurium, and at the dose of 10 mg/ml, CLPS1000 possesses the therapeutic effect for the infection of S. typhimurium in mice.

요약

본 연구는 황색, 금관화, 그리고 백설쪽 혼합분말(1:1:1)의 배양액 주출물(CLP1000)과, 이에 dioctahedral smectite를 혼합한 혼합액(CLPS1000)의 살모넬라에 대한 항균효과를 평가하기 위해 수행되었다. CLP1000과 CLPS1000은, 0.5 mg/ml 늑도에서 S. typhimurium에 대한 항균효과를 보이지 않았으나, 1.0 mg/ml의 늑도에서는 S. typhimurium에 대한 항균활동이 관찰되었다. BALB/c 마우스를 이용한 살모넬라 감염시험에서 Smectite, CLP1000 그리고 CLPS1000을 각각 10 mg/ml 늑도로 12일 동안 두여한 결과, 사망률이 각각 90%, 90% 그리고 70%를 보여, CLPS1000이 마우스 살모넬라중증에 강력한 효과를 갖고 있는 것으로 나타났다.

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References


