The Antifungal Activity of *Phellodendron chinense* Schneid Ethanol Extract Against Trichophyton Mentagrophytes in Rabbits

Xiao Chenwen, Ji Quanan, Bao Guolian, Liu Yan, Wei Qiang

Institute of Animal Husbandry and Veterinary Science, Zhejiang Academy of Agricultural Sciences, Hangzhou, Zhejiang 310021, PR China, Phone: +86-571-86400373; Fax: +86-571-86400836
Correspondence e-mail: xiaochenwen.1979@yahoo.com.cn

ABSTRACT

Trichophyton mentagrophytes disease in rabbits could induce hair removal, scab, dandruff and itching, the disease spreads quickly and damage rabbit industry seriously. The bark from the Phellodendron tree has been used as traditional Chinese medicine for curing gastroenteritis, abdominal pain or diarrhea and various inflammatory diseases including arthritis and dermatophytosis. The present study was undertaken to evaluate the antifungal effects of ethanol extract of *Phellodendron chinense* (PAEE) against *Trichophyton mentagrophytes in vivo* and *in vitro*. The milled dried bark was extracted with 75% ethanol at 80°C three times under reflux for 2 h. The combined solution was concentrated with a rotary evaporator at 45° to a concentration of PAEE (2 g/ml). Minimum inhibitory concentration (MIC) was measured using agar-diffusion method with little modification. In brief, serial volume of PAEE or clotrimazole (as the positive control) (0, 0.5, 1, 1.5, 2, 2.5 or 3 mg dissolved in 0.2 ml DMSO) were gently mixed with 100 ml of Tryptic soy agar (TSA), poured into sterile petri dishes and incubated at 45 °C for 15 min. The final concentration of the PAEE in TSA was 0.00, 0.01, 0.02, 0.03, 0.04, 0.05, 0.06 g/ml. Then, 1.0×10^6 CFU/ml eumycete suspension was inoculated and incubated for 72 h. The MIC was taken as the lowest concentration of PAEE or clotrimazole to inhibit growth of the fungus. Each experiment was carried out in duplicate. *In vivo* antifungal assay, 30 New Zealand white rabbits, 31 days old, weighing 400-450 g were divided into five groups consisting of six rabbits in each by simple random method, a suspension (1 ml, 1.0×10^6 cells) of *T. mentagrophytes* was applied to the marked area using a sterile pipette-tip and rubbed thoroughly for 3 consecutive days (1, 2, 3 d). Various concentration of PAEE (group PA1, PA2, PA3) formulations or clotrimazole (group PC) or distilled water (group NC) were applied topically on day 4 and continued up to day 7 (4, 5, 6, 7 d). The lesions were evaluated from the day of post-infection to 10th day continuously. The clinical evaluation was consisted of a semi-quantitative score. During quantitative analysis, the total alkaloids in PAEE was found 7.58±0.46 mg/ml. *In vitro* studies, the MIC values for PAEE and clotrimazole were 1.5% (0.03g/ml and 0.02 mg/ml, respectively). The influence of different doses of PAEE on the growth of *T. mentagrophytes* was also detected by dry weight determination. In addition, transmission electronic microscopy was performed to observe the effect of PAEE on cell ultrastructure and it showed that PAEE destroyed the cell membrane of *T. mentagrophytes*. Furthermore, dermatophytosis infection model in rabbit with *T. mentagrophytes* was established for investigating the effect of PAEE (PA1, PA2, PA3) *in vivo*. The efficacy of each group was PA1 (81.9%), PA2 (87.5%), PA3 (86.1%), PC (83.3%), NC (0.00), respectively. All treatment groups showed the inhibitory effects of PAEE against *T. mentagrophytes*. It can be concluded that PAEE has significant of antifungal activity and could be used as a treatment to *T. mentagrophytes* in rabbits.

Key Words: Phellodendron Chinense, Trichophyton Mentagrophytes, Antifungal, Rabbit