Veterinary Immunology and Immunopathology 173 (2016) 34–38

Contents lists available at ScienceDirect

Veterinary Immunology and Immunopathology

journal homepage: www.elsevier.com/locate/vetimm

Short communication

Effects of dietary supplementations with the fibrous root of *Rhizoma Coptidis* and its main alkaloids on non-specific immunity and disease resistance of common carp

Xia Zhou[a], Yaozong Peng[a], Ling Li[a], Kai He[a], Tao Huang[a], Shaoxia Mou[a], Min Feng[a], Bing Han[b], Xiaoli Ye[b,c,**], Xuegang Li[a,c,*]

[a] School of Pharmaceutical Sciences, Southwest University, Chongqing 400715, China
[b] School of Life Sciences, Southwest University, Chongqing 400715, China
[c] Chongqing Productivity Promotion Center for Modern Chinese Medicine, Chongqing 400715, China

ARTICLE INFO

Article history:
Received 11 September 2015
Received in revised form 16 March 2016
Accepted 25 March 2016

Keywords:
Fibrous root of *Rhizoma Coptidis*
Non-specific immunity
Disease resistance
Aeromonas hydrophila

ABSTRACT

The effects of fibrous root of *Rhizoma Coptidis* (FRC) and its main alkaloids on non-specific immunity and disease resistance of common carp were investigated. The fish were randomly assigned to seven groups: normal control group (NC), groups treated with 12.5 g/kg FRC (FRC-L), 25 g/kg FRC (FRC-M), 50 g/kg FRC (FRC-H), 0.78 g/kg total alkaloids (TA), 0.78 g/kg berberine (BBR), and 0.78 g/kg coptisine (Cop), respectively. Results showed that the activities of myeloperoxidase, lysozyme and respiratory burst were significantly elevated after treated with FRC-M, FRC-H, TA, BBR and Cop, and the complement C_3 level and phagocytic activity were significantly increased in FRC-M, TA and BBR treated groups compared with NC group. The real-time PCR analysis indicated that FRC, TA, BBR and Cop could up-regulate the mRNA expression of IL-1β, TNF-α, lysozyme-c and C_3, but down-regulate that of IL-10 in the head kidney of common carp. Besides, FRC-M, FRC-H, TA, BBR and Cop significantly enhanced the survival rate of common carp infected with *Aeromonas hydrophila*, when compared to NC group. It was concluded that the FRC could enhance the non-specific immunity and disease resistance of common carp and the main alkaloids might contribute to these effects.

1. Introduction

Common carp is an important commercial freshwater fish species in aquaculture industry. However, as the increasing of breeding density, the outbreak of common carp diseases is more and more frequently. In recent years, many natural herbs such as *Astragalus root* (Wang et al., 2009) and *Eclipta alba leaf* (Christybapita et al., 2007) have been applied to prevent fish diseases due to their little side effects and immune-improving efficiency.

Rhizoma Coptidis (RC), the root of *Coptis chinensis* Franch, has been widely used in veterinary clinics. Pan et al. (2013) reported that 2% of RC extracts could enhance the leukocyte phagocytic and lysozyme (LZM) activities and the survival rate of red drum. In China, RC is mainly used to treat human diseases as a famous traditional Chinese medicine with a relatively high price, therefore, massive application of RC in aquaculture will be a heavy burden for farmers. To solve this problem, we focused on the utilization of its fibrous root (FRC), a by-product in the processing of RC, which was always abandoned in the field by farmers. Surprisingly, we found that the content of total alkaloids in FRC is about 1/3 of RC and FRC exerts similar pharmacological functions to RC, indicating a potential to be a new veterinary drug. Now we have completed its clinical researches on animals, and is currently undergoing the application and approval process for new veterinary drugs named as "fibrous root of *C. chinensis* Franch" and "fibrous root powder of *C. chinensis* Franch" (registration No. 0705001577 and 0705001578). In order to detect whether FRC has the ability to increase non-specific immunity and disease resistance, common carp were used as the animal model and the effects of the FRC and its main alkaloids on non-specific immunity and disease resistance of common carp were investigated.

* Corresponding author at: School of Pharmaceutical Sciences, Southwest University, Beibei, Chongqing 400715, China.
** Corresponding author at: School of Life Sciences, Southwest University, Chongqing 400715, China.
E-mail address: xuegangli@swu.edu.cn (X. Li).

http://dx.doi.org/10.1016/j.vetimm.2016.03.014
0165-2427/

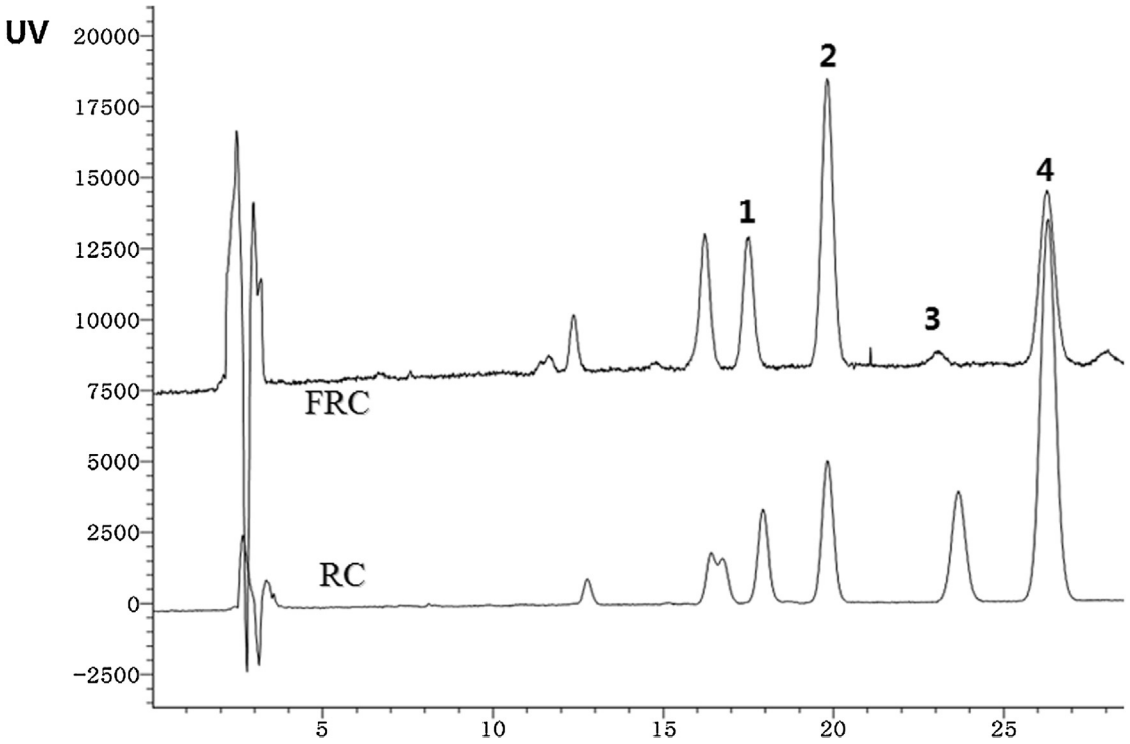

Fig. 1. HPLC chromatograms of fibrous root of *Rhizoma Coptidis* (FRC) and *Rhizoma Coptidis* (RC). 1–4 represented the epiberberine, coptisine, palmatine and berberine, respectively.

2. Materials and methods

2.1. Chemicals

The standard of berberine (BBR) was purchased from Chengdu Biopurify Phytochmicals Ltd. (Chengdu China). Total alkaloids (TA) were the mixture of berberine (BBR), coptisine (Cop), epiberberine (Epi) and palmatine (Pal) isolated from FRC according to their original percentage in FRC (Chen et al., 2012; Liu et al., 2010). Nitroblue tetrazolium (NBT) was purchased from Beijing Notlas Biotech Co., Ltd. (Beijing, China). *Micrococcus lysodeikticus*, lysozyme, Giemsa and complement C_3 kits were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). *Aeromonas hydrophila* (XS91-4-1) was obtained from Chinese Academy of Sciences (Beijing, China). Other chemicals used were supplied by Dingguo Bio-Technology Company (Beijing, China).

2.2. Characterization of FRC

Specimens of the FRC and RC were collected from the Good Agricultural Practice Base in Shizhu, Chongqing, China. Collection was performed in October 2013 and was authenticated by Prof. Xuegang Li. The alkaloids of FRC and RC (Fig. 1) were analyzed by high-performance liquid chromatography (HPLC) (Wagner et al., 2011; Ning et al., 2015). Before used, FRC and RC were dried and ground into powder.

2.3. Experimental diets

According to the improved methods (Rao et al., 2006), 780 mg of TA, BBR and Cop were added into 1 kg of normal diet ingredients to get TA, BBR and Cop supplemented diets, respectively (marked as TA, BBR and Cop diets). For FRC diets, 12.5 g/kg, 25 g/kg and 50 g/kg of FRC powder were supplied to the normal diet ingredients for obtaining low, medium and high dosage of FRC supplemented diets, respectively (labeled as FRC-L, FRC-M, FRC-H diets). Briefly, normal diets ingredients and supplements above were mixed thoroughly by a drum mixer, then added distilled water to prepare uniform sized pellets using a laboratory pelletizer. After dried at 50 °C for 24 h, the pellets were packed, sealed, labeled and stored at 4 °C.

2.4. Experimental design

Healthy non-vaccinated common carp weighing 150 ± 20 g were obtained from Sichuan Aquatic School (Chongqing, China). The fish were allowed to acclimatize to the new recirculation freshwater system of glass tanks at 25 °C and fed the basal diets for 7 days. Then, fish were randomly distributed into seven groups (3 tanks per group, 16 fish per tank): NC, FRC-L, FRC-M, FRC-H, TA, BBR and Cop, which were fed with basal diets, FRC-L, FRC-M, FRC-H, TA, BBR and Cop supplemented diets, respectively, twice a day (at 09:00 and 18:00) for 21 days at the rate of 1% of body weight. At the end of the experiments, six fish per tanks were anaesthetized to collect the serum and head-kidneys, and the rest ten fish in each tank were injected with 0.2 ml of *A. hydrophila* to start an experimental challenge trial.

2.5. Isolation of head kidney-derived macrophages

Macrophages were isolated from the head kidney of common carp according to the method described by Secombes (1990) and Joerink et al. (2006) with minor modifications. Briefly, after narcotized, the head-kidney was removed aseptically and gently pushed through a 100 μm nylon mesh with L-15 culture medium containing 5% fetal calf serum, 10 U/ml heparin, 100 U/ml penicillin and 100 mg/ml streptomycin. Washed cells at 600*g* for 10 min and suspended in L-15 culture medium. Then, cell suspensions were layered on a 34%/51% Percoll discontinuous density gradient and centrifuged at 400*g* for 30 min at 4 °C without using the brake. Then, the cells at the medium interface were collected to obtain enriched