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Original article

Effects of *Coptidis Rhizoma* on Cell Cycle, DNA Damage, and Apoptosis in L929 Murine Fibroblast Cells

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ABSTRACT

Objective *Coptidis Rhizoma* (CR), a widely used traditional Chinese herbal medicine, is commonly believed to be non-toxic. However, little is known about its cytotoxicity and relevant mechanisms at cellular and genetic levels. The present study was conducted to explore the cytotoxicity of CR and its mechanisms related to cell cycle arrest, DNA damage, cell apoptosis, and mitochondrial membrane potential in L929 murine fibroblast cells. **Methods** The cells were cultured and treated with different concentration of CR aqueous extract for 24 h. Cell viability was determined by CCK-8 method, morphological changes, and mitochondrial membrane potential were observed with an inverted microscope, cell cycle and cell apoptosis were examined by flow cytometry and DNA damages were detected by comet assay. **Results** Our results showed that cell viability was significantly decreased in a dose-dependent manner when concentration was higher than 0.2 mg/mL. A concentration above 1 mg/mL altered the cells morphology. Each DNA damage indicator score increased in the groups with the concentration of above 0.1 mg/mL. Cells at G_2/M phase, cell apoptosis and mitochondrial membrane potential changed in the 2 mg/mL group. **Conclusion** Overall, our study suggests that CR at a high dosage exhibits cytotoxicity on L929 cells, which is likely to be the consequences of cell cycle arrest, DNA damage, cell apoptosis and mitochondrial membrane potential reduction.

Key words

apoptosis; cell cycle; *Coptidis Rhizoma*; cytotoxicity; DNA damage; mitochondrial membrane potential

1. Introduction

Coptidis Rhizoma (CR), the dried rhizome of *Coptis chinensis* Franch., *C. dletoidea* C. Y. Cheng et Hasiao, and *C. teeta* Wall., serves as the medicinal part with bitter taste, cold nature, and tropism to the channels of heart, liver, stomach, and large intestine. It belongs to the antipyretic category because it plays a role in clearing away the damp-heat, purging fire away from the heart, and eliminating toxic materials from the body. Moreover, CR is used as a common traditional herbal drug for treating infectious diseases (Ma, 2013). The main components of CR are isoquinoline alkaloids (Qiu, 2012; Qing et al, 2016), of which berberine is the highest, followed by coptisine, palmatine, epiberberine, and so

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on (Figure 1). Previous pharmacological studies have demonstrated that CR has multiple bioactivities, such as an increase in platelet count, neuro-protective and immune-modulated (Yu et al, 2006), antimicrobial (Choi et al, 2007), anti-inflammatory (Lu et al, 2011), antineoplastic (Tang et al, 2009), anti-Alzheimer, anti-oxidant (Jung et al, 2009), hepatoprotective (Ye et al, 2009), analgesic (Tjong et al, 2011), and antihyperglycemic (Chen et al, 2012) effects. CR has been widely used in clinic for a long time. *Treatise on Exogenous Febrile Disease*, a classical work by Zhong-jing Zhang (a famous doctor in Han dynasty), describes 12 types of prescriptions containing CR. There are at least 50 contemporary Chinese medicines containing CR. According to the *Ministry of Foreign Trade and Medicine Companies' Statistic Data*, the demand of CR in the market reached 2000 tons by 2006. With the discoveries of various efficacies of CR and remarkable curative effects in the treatment of various disorders, the clinical use of CR is quickly expanding.

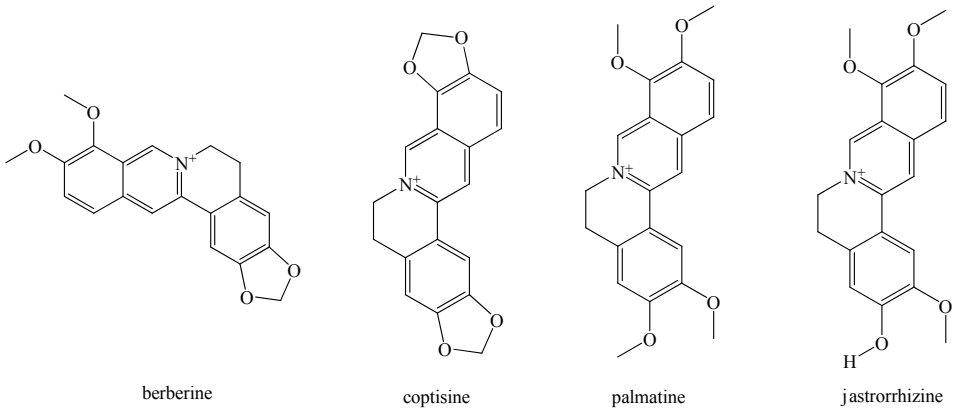

Figure 1 Molecular structures of four primary active constituents from CR

It is widely accepted that traditional Chinese herbal medicines are natural with very few side effect. In addition, CR has been regarded as non-toxic according to its top grade in *Shennong's Herbal Classic*, as well as its essential role in many detoxification prescriptions. But since 1978, there have been some suspected cases reported in Singapore that pregnant women or newborns who took CR or its active principle berberine manifested the defects of glucose-6-phosphat dehydrogenase (G6PD), leading to the neonates suffering hemolytic jaundice or kernicterus. Many studies have been conducted regarding the issue whether CR and berberine are toxic. Yeung et al (1990) and Yang (2000) demonstrated that CR could aggravate the risk of neonatal jaundice in related experiments in 1990 and 2000, respectively. In 2001, Yu et al (2001) implemented a clinical treatment of diabetes with 8−18 g CR grinds powder capsules, adverse reactions emerged, such as nausea, hypoglycemia, and diarrhea. Li et al and Qiu et al found that rats receiving high doses of CR decoction orally showed the dysfunction of gastric mucosal barrier and decrease of prostaglandin E2 (Li et al, 2007; Qiu et al, 2004). Previous reports have also demonstrated that CR and its products induce adverse reactions, including drug eruption, allergies, pancytopenia, dizziness, palpitation, shortness of breath, and joint pain (Feng, 2004; Zhang et al, 2003). These results all strongly suggest that CR has certain toxicity. Therefore, it is reasonable to speculate that CR is not as safe as commonly believed in some cases. However, it remains unclear the toxicity and its relevant mechanisms of CR, especially those at the cellular and genetic levels.

In this study, we used L929 cells line to perform toxicity testing, aiming to explore the cytotoxicity of CR and the related mechanisms. Our study would provide a theoretical basis for its safe applications and allow a rational evaluation of the pharmacological effects.

2. Materials and methods

2.1 Plant materials

Coptidis Rhizoma (CR), national drug standard substance, the dry powder of *Coptis deltoidea* C.Y. Cheng et Hsiao (the batch number is 120913-201310) was purchased from the National Institutes for Food and Drug Control (Beijing, China). CR was extracted as previously described (Ma et al, 2010) with minor modifications based on traditional methods. CR was extracted in boiling distilled water for three times. After filtration, the mixed filtrate was concentrated by a rotary evaporator to 0.1 g/mL at 45 ^{o}C, then the aqueous extract was sterilized by 0.22 μm microporous membrane and saved at 4 ^{o}C. The CR aqueous extract (0.1 g/mL) will be diluted with cell culture medium to the required concentrations for experiments.

2.2 HPLC analysis

Determination of CR contents was performed using HPLC (Agilent 1100 Series: Agilent Technologies Ltd., USA) with a Diamonsil C_{18} column (150 mm × 4.6 mm, particle size 5 μm). In HPLC experiments, the mobile phase was cetonitrile-water solution of 50 mmol/L potassium dihydrogen phosphate (50:50, pH 4.0, containing 15 mmol/L lauryl sodium sulfate), gradient elution was in a mixture of potassium phosphate monobasic (50 mmol/L), and acetonitrile was at column temperature of 30 ºC, with a flow